POST-TREATMENT FOR ANAEROBIC EFFLUENTS

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SUMMARY

The post-treatment study for anaerobic effluents was carried out in 2 phases with different focuses.

In phase 1, the focus was on organic removal and nitrification performance of post-treatment for anaerobic effluents. Performances of Conventional Activated Sludge (CAS) and Membrane Bioreactor (MBR) were compared at HRTs of 8 and 4 h; with anaerobic pre-treatment systems operated at HRTs of 16 and 6 h, respectively.

All CAS and MBR systems investigated were able to produce effluents of consistently good quality (less than 50 mg/L in tCOD and less than 10 mg/L in tBOD) that were able to meet the discharge requirement to controlled watercourse (tCOD < 60mg/l and tBOD < 20mg/l) of Singapore (NEA, 2005). MBRs outperformed CAS for both COD and BOD removals as performance of MBRs are independent on the settleability of biomass.

MBR was able to achieve complete solid-liquid separation, therefore no SS was observed in the effluent of MBR. The CAS were able to achieve effluent SS concentration of less than 30 mg/L to meet Singapore standard for discharge to controlled watercourse (NEA, 2005)

The ratios of COD/BOD were found to be above 3, suggesting that biodegradation could be slow or difficult. Despite of this, both CASs and MBRs were still able to produce good quality effluents.

A near complete nitrification performance was observed most of the time for all the CASs and MBRs operated at both 8- and 4- h HRT.

In phase 2, the focus was shifted to nitrogen removal through biological nitrification-denitrification for anaerobic effluents. As carbon source is an important factor that affects the rate of denitrification, different percentages of raw sewage were added to vary carbon concentration in anaerobic effluents. The chosen percentages of raw sewage addition were 0%, 25% and 50%. Rates of denitrification were compared at different COD/N ratios and the optimal COD/N ratio recommended to achieve effective nitrogen removal for anaerobic effluents.

CASs could achieve more than 85% tCOD removal while more than 90% removal was achievable by MBRs for all the 3 different percentage of sewage additions.

The nitrogen removal efficiency had improved tremendously from 20% to approximately 70% with the introduction of anoxic tanks for post-treatment systems treating the UASB and AF effluents. However, only about 50% removal was achieved in post-treatment of the effluents from the anSBR.

TN removal efficiencies for post-treatments of the effluents of the UASB and AF were similar to the operating condition when 0% of raw sewage was added. However, TN removal efficiencies of the post-treatment of the anSBR effluent improved tremendously by 16 and 19% for the CAS and MBR, respectively.

More than 70% TN removal efficiencies were achieved in all the post-treatment systems after addition of 50% sewage. However, the nitrification performance deteriorated with traces of NH_4^+-N found in effluents of both the CASs and MBRs.

To optimise the post-treatment process for anaerobic effluent, 50% raw sewage addition is the most ideal as the removal performances for nitrogen and organics were relatively high. In addition, it reduced the capacity of the pre-treatment while maximised the use of aeration in the post-treatment.

NOMENCLATURE

CAS	Conventional activated sludge
MBR	Membrane bioreactor
UASB	Upflow anaerobic sludge blanket
AF	Anaerobic filter
anSBR	Anaerobic sequencing batch reactor
MF	Microfiltration
UF	Ultrafiltration
RO	Reverse osmosis
TDS	Total dissolved solids
UASB	Upflow anaerobic sludge blanket
AF	Anaerobic filter
AAFEB	Anaerobic attached film expanded bed
AFBR	Anaerobic fluidized bed reactor
anSBR	Anaerobic sequencing batch reactor
ABR	Anaerobic baffled reactors
OLR	Organic loading rates
RBC	Rotating biological contactor
TKN	Total Kjeldahl nitrogen
BOD	Biochemical oxygen demand
TMP	Transmembrane pressure
NH_4^+-N	Ammonium
NO_2^--N	Nitrite
NO_3^-N	Nitrate
DO	Dissolved oxygen
NOD	Nitrogeneous oxygen demand
C/N	Carbon to nitrogen ratio
F/M	Food to microorganism ratio
HRT	Hydraulic retention time (h)
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solid

sCOD	Soluble Chemical Oxygen Demand
SRT	Solid retention time (d)
tCOD	Total Chemical Oxygen Demand
TOC	Total Organic Carbon
TSS	Total suspended solid (mg/L)
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solid (mg/L)

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CHAPTER ONE INTRODUCTION

"Access to water for life is a basic human need and a fundamental human right. Yet in our increasingly prosperous world, more than 1 billion people are denied the right to clean water and 2.6 billion people lack access to adequate sanitation. Close to half of all people in developing countries suffering at any given time from a health problem caused by water and sanitation deficits." (United Nations Development Programme, 2006).

Wastewater is used water. It comprises of liquid waste discharged by domestic residences, commercial properties, industry and agriculture. Wastewater can encompass a wide range of potential contaminants and concentrations. When left untreated, it will pose a great danger to human health as well as causing damage to the ecosystem.

Nature has an amazing ability to cope with small amounts of wastes and pollution. However, population growth, urbanization, industrial development and the needs of agriculture are driving up the amount of pollutants in wastewater that has exceeded the limits nature can cope. Hence, treatment is essential to reduce pollutants to a level that nature can handle.

Over the years, numerous processes have been developed and are available to treat wastewater depending on the type and extend of contamination. They include physical, chemical and biological treatment processes. For wastewater containing organic matter,

aerobic biological treatment has been a popular option. Almost all medium and large wastewater treatment plants were designed with an aerobic reactor as the main unit of achieving efficient organic matter removal until the eighties. Traditionally, anaerobic biological treatment was mainly applied to sludge digestion.

However, in 1970s, the attractiveness of aerobic methods was reduced due to the steep increase in energy prices and contributed to redirecting research efforts towards energy-saving alternatives like anaerobic treatment (van Haandel & Lettinga, 1994).

1.1 Anaerobic wastewater treatment

Anaerobic wastewater treatment is the biological treatment of wastewater without the use of air or elemental oxygen. It is a multi-step biological process in which organic matter is converted partially to gas mixture of methane and carbon dioxide.

Anaerobic wastewater treatment is one of the oldest biological wastewater treatment processes which was first studied more than a century ago (McCarty, 1981). The first application of anaerobic digestion for sewage treatment dates back to about 1860, with the development of a simple air-tight chamber by Mouras in France (McCarty, 2001), referred to as septic tank.

Although anaerobic wastewater treatment has been used since the late 19th century, it is considered to be an unstable, inefficient and slow process. In addition, experience with anaerobic digestion of sludge, where only 50% reduction of solids was possible, even at long stabilization times, led researchers to lose interest in the application of

anaerobic processes for the treatment of liquid wastes. Hence, anaerobic treatment was applied mainly to the digestion of sludge in its early application.

Yet, the belief that anaerobic treatment was an inefficient process was a fallacy related to experiences with sludge digestion, where most of the organic material being treated is not readily susceptible to biological degradation (McCarty, 1964). Besides, the drawback for wastewater treatment was noted based on past experiences with the conventional suspended growth, completely mixed anaerobic reactors.

The major limitations of anaerobic process are the low growth yield and long doubling times of the microorganisms, especially for those involved in the acetogenic and methanogenic reactions. To achieve efficient anaerobic treatment of wastewater, the maintenance of a high population of biomass in the reactor would be necessary to compensate for the slow growth rate of anaerobic organisms which can result in system failure if a high loss of biomass in the effluent occurs.

The difficulty was overcome with the development of high-rate anaerobic biological processes which can achieve high levels of biomass in anaerobic reactors and long solid retention time (SRT) while maintaining short hydraulic retention time (HRT).

In 1970s, anaerobic treatment started to gain more attention for research and technology development due to the rapid escalation of energy costs as well as the increased environmental awareness. This resulted in a better understanding of the complex microbial processes and several improvements of the technology.

As a result, several anaerobic high-rate reactors were developed. They include anaerobic filter (Young and McCarty, 1967), upflow anaerobic sludge blanket (Lettinga *et al*., 1980), fluidized and expanded bed reactors (Switzenbaum and Jewell, 1980) and anaerobic sequencing batch reactor (Dague *et al*., 1967, 1970). The development has resulted in increased efficiency and utilization of anaerobic processes for the treatment of various liquid wastes.

Since then, anaerobic treatment has been applied extensively for the treatment of industrial wastewater that includes the pulp and paper industry (Bajpai, 2000), food industry (Matteson and Jenkins, 2007), breweries (Parawira *et. al.*, 2005) and distilleries (Akarsubasi *et. al.*, 2006).

However, anaerobic processes were seldom applied to the treatment of low-strength wastewater with COD less than 1000mg/l, as there was a common perception that anaerobic processes were not capable of achieving efficient organic destruction due to the low utilization rate of the substrate at low concentrations. Moreover, the methane produced will be minimal and insufficient to supply heat source for efficient performance of anaerobic digestion at a relatively high operating temperature.

Yet, with the better understanding of complex microbial in anaerobic process and development of technology, there is a growing interest in the application of anaerobic process for the treatment of low-strength wastewaters (Mergaert et al., 1992), especially in tropical regions of the world. Many researches had been conducted and the technology has been successfully implemented in tropical countries such as Brazil, Columbia and India (Seghezzo *et. al.*, 1998; Nobuyuki *et. al.*, 2006).

1.2 Post-treatment of anaerobic effluents

Despite of the relatively good removal efficiencies of anaerobic treatment, effluents from anaerobic reactors can rarely comply with the effluent discharge standards. Besides the remaining fraction of particulate and soluble organic matter; nutrients and pathogens are the other main important constituents that deserve attention. They are not removed adequately in the most commonly used anaerobic reactors. Therefore, post-treatment is essential to polish the anaerobic effluents before final discharge or further treatment for various usages.

Anaerobic treatment can remove up to 70% of COD; therefore reduce the carbon concentration greatly. Yet, nitrogen concentration is unaffected. As a result, the carbon to nitrogen ratio is altered after the treatment. In order to achieve an efficient nitrogen removal, sufficient carbon source is required. Hence, supplement carbon source might need to be added to supply enough electron donors for maximum denitrification to occur.

With the aim to reach better process stability and performance efficiency, several researches focused on combinations of the anaerobic and aerobic processes were conducted. The combined process is more sustainable than the conventional aerobic systems because of low excess sludge production and energy input.

Various aerobic treatment systems have been proposed for post-treatment; such as activated sludge (Haandel and Lettinga, 1994), down-flow hanging sponge (Tawfik *et.*

al., 2006), rotating biological contactor (Tawfik *et. al*., 2002, 2005) and integrated duckweed and stabilization pond system (Peter *et. al*., 1999).

1.3 Membrane bioreactor (MBR)

With increasing emphasis being placed on the public health and the environment, more stringent measures were imposed on the quality of treated effluent. In addition, due to the problems of water scarcity and a rapidly increasing world population, new water sources have to be explored. While seawater desalination has gained much popularity, the cities that are located in the arid regions or far away from the sea would not be able to benefit from seawater desalination. Thus reclaiming domestic wastewater would be an attractive alternate option for water augmentation. As about 80 to 90 % of public water supply in most cities will end up as domestic wastewater, domestic wastewater can provide a very reliable water source with its volume fluctuates slightly throughout the year (Sadr Ghayeni *et al.*, 1998).

The reclamation of domestic wastewater is achieved first by biological treatment such as conventional activated sludge (CAS). The treated effluent from CAS will then undergo tertiary filtration by microfiltration (MF) or ultrafiltration (UF). The filtrate will lastly pass through the reverse osmosis (RO) for removal of dissolved solids (DS). In Singapore, the effluents produced by the RO process are used as potable water via indirect potable reuse application or ultra-pure water for industrial usage.

Effective solid-liquid separation of mixed liquor is an essential step in the CAS process as it has a major influence on effluent quality. Traditionally, this has been accomplished by a gravitational settling tank.

In recent year, the application of membrane technology especially in conjunction with biological systems has attracted a great attention in wastewater treatment along with the progress of membrane manufacturing technology (Brindle and Stephenson, 1996). The adaptation of membranes coupled with an aerobic biological process offers the possibility of developing an efficient wastewater treatment process being capable of completely retaining biomass in the bioreactor and producing a high quality effluent.

Membrane bioreactor (MBR) system incorporates the activated sludge process into one single reactor. Hence, conventional gravitational settling tank for the separation of the treated water from the sludge is eliminated. In addition, MBR can operate at a high mixed liquor suspended solids (MLSS) concentrations that improve the treatment efficiency and is directly translated into a reduction in reactor size. Thus, there is a great reduction in the overall land area of the system as compared to the conventional treatment plant. Moreover, the introduction of submerged membranes has reduced the power consumption of MBR significantly (Gander *et al.*, 2000). This helps to increase the application potential of membrane in wastewater treatment in recent years.

However, in spite of the many advantages of the MBR, there is a restriction on the widespread application of MBR technology due to the inherent cost of membranes as well as energy costs resulted from high aeration and pumps requirements.

MBR can retain not only bacteria and colloidal material which are larger than the cut-off point of membranes, but also high molecular weight components either present in the wastewater or released through bacterial lyses. High MLSS is able to be retained in the reactor and provide good performances; however this also causes high viscosity of the reactor content. As a consequence, a high aeration capacity is required to counteract fouling of the membrane and to account for heavily decreased oxygen transfer coefficients

1.4 Project objectives

In this study, aerobic treatment was proposed for the post-treatment of anaerobic effluents. Performances of two different systems – CAS and MBR were compared. The study aimed to access the feasibility of replacing CAS with MBR to achieve better organic and nitrogen removal for anaerobic effluents. While MBR has been proven to be a viable technology, the higher cost involved due to high aeration capacity needed and higher energy has limited the widespread use of MBR. Therefore, the study also included the comparison of economical operation of MBR as compared to CAS. To achieve these, the study was carried out in two different phases.

In phase 1, the focus was on organic removal and nitrification performance under different HRTs. Both laboratory-scale CAS and MBR were started simultaneously with similar operating conditions. Two different HRTs of 8 and 4 hours were studied. The specific objectives for this phase of the study were to:

- Investigate the organic removal and nitrification performance under different HRTs
- Study the mixed liquor concentration and sludge yield of CAS and MBR
- Determine the accumulation of SMP and EPS production of CAS and MBR
- Study the membrane fouling phenomenon

In phase 2, the focus was on nitrogen removal for the anaerobic effluents. Anoxic tanks were added to both CAS and MBR. Both anoxic tanks were seeded with sludge drawn from the respective system. Common HRT was used. The effect of COD/N ratios on nitrogen removal with the addition of raw sewage was studied. The specific objectives for this phase of the study were to:

- Investigate the nitrogen removal performance of CAS and MBR
- Study the effect of COD/N ratios on the nitrogen removal performance
- Study the microbial community in CAS and MBR under different COD/N conditions

CHAPTER TWO LITERATURE REVIEW

2.1 Anaerobic treatment

Anaerobic wastewater treatment consists of a series of microbiological processes that convert organic compounds into a small amount of sludge and a large amount of biogas (methane and carbon dioxide) in the absence of oxygen molecule.

2.1.1 Biochemistry of anaerobic process

In anaerobic biological treatment systems, a consortium of diverse and yet closely dependent group of bacteria are involved in the transformation of complex organic compounds into methane and carbon dioxide. The overall reaction is expressed as follows:

$$\text{Organic Matter} \rightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S \quad (2.1)$$

The biological degradation of complex organic compounds takes place in several consecutive biochemical phases (chain-reaction) each performed by different groups of specialized bacteria. Several intermediate products are continuously generated and processed further immediately upon generation. It is important to realize that all phases have to occur at matching rates in order to avoid build-up of intermediate products.

Novaes (1986) classified the metabolic processes and microbial groups involved in anaerobic treatment into five groups (as presented in Figure 2.1). The five groups are: (1) Fermentative (hydrolytic) bacteria, (2) H_2-producing acetogenic bacteria, (3) H_2-consuming acidogenic or homoacetogenic bacteria, (4) CO_2-reducing methanogenic bacteria, and (5) Acetoclastic methanogenic bacteria. The overall conversion process could be distinguished into four main phases, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Stronach *et al*., 1986; Marty, 1986).

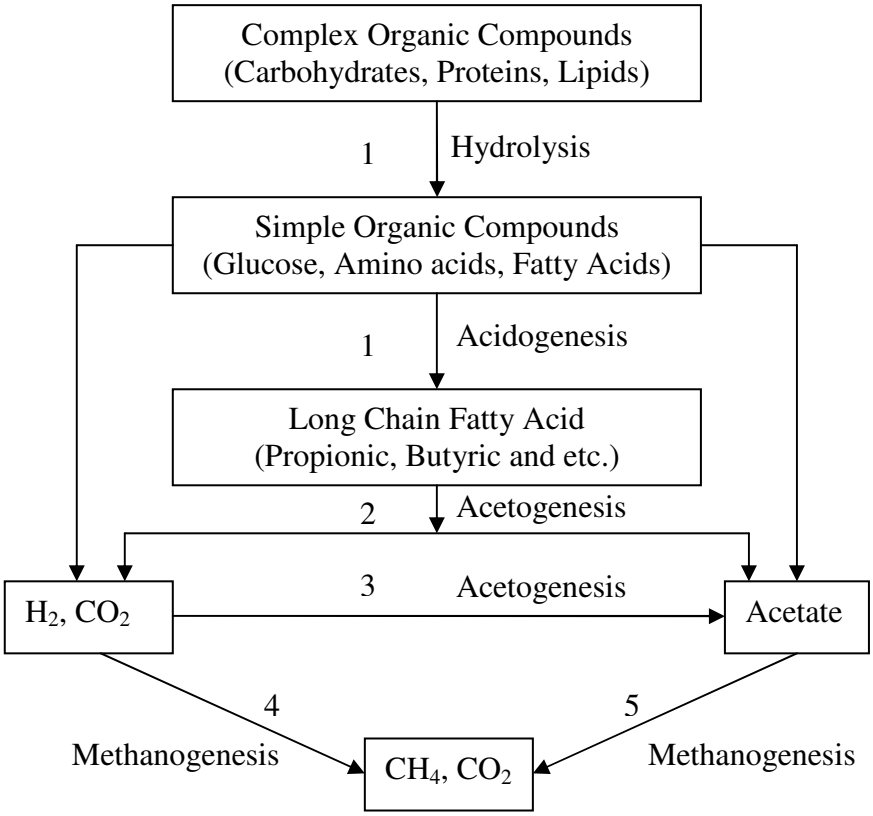


Figure 2.1 Metabolic steps and microbial groups involved in anaerobic treatment: (1) Fermentative (hydrolytic) bacteria, (2) H_2-producing acetogenic bacteria, (3) H_2-consuming acidogenic or homoacetogenic bacteria, (4) CO_2-reducing methanogenic bacteria and (5) Acetoclastic methanogenic bacteria (Novaes, 1986)

2.1.2 Advantages and drawbacks of anaerobic treatment

Anaerobic processes have been used to treat concentrated domestic and industrial wastewater for over a century (McCarty & Smith, 1986) with the simplest, oldest, and most widely used process being the septic tank (Jewell, 1987). Despite of its advantages of low sludge production and biogas generation, it was not used widely due to its long start-up period required and the inability to remove pathogens and nutrients as compared to aerobic treatment. Hence, aerobic process has been a popular choice of main treatment unit for wastewater in the past century. The advantages and drawbacks of anaerobic wastewater treatment are summarized in Table 2.1.

Table 2.1 Advantages and drawbacks of anaerobic wastewater treatment (Adapted from Seghezzo *et al.*, 1998)

Advantages	
High efficiency	Good removal efficiency can be achieved in the system, even at high loading rates and low temperatures.
Simplicity	The construction and operation of these reactors is relatively simple.
Flexibility	Anaerobic treatment can easily be applied on either a very large or a very small scale.
Low space requirements	When high loading rates are accommodated, the area needed for the reactor is small.
Low energy consumption	As far as no heating of the influent is needed to reach the working temperature and all plant operations can be done by gravity, the energy consumption of the reactor is almost negligible. Moreover, energy is produced during the process in the form of methane.
Low sludge production	The sludge production is low, when compared to aerobic methods, due to the slow growth rates of anaerobic bacteria. The sludge is well stabilized for final disposal and has good dewatering characteristics. It can be preserved for long periods of time without a significant reduction of activity, allowing its use as inoculum for the start-up of new reactors.

Low nutrients and chemicals requirement	Especially in the case of sewage, an adequate and stable pH can be maintained without the addition of chemicals. Macronutrients (nitrogen and phosphorus) and micronutrients are also available in sewage, while toxic compounds are absent.
Drawbacks	
Low pathogen and nutrient removal	Pathogens are only partially removed, except helminth eggs, which are effectively captured in the sludge bed. Nutrients removal is not complete and therefore a post-treatment is required.
Long start-up	Due to the low growth rate of methanogenic organisms, the start-up takes longer as compared to aerobic processes, when no good inoculum is available.
Possible bad odors	Hydrogen sulphide is produced during the anaerobic process, especially when there are high concentrations of sulphate in the influent. A proper handling of the biogas is required to avoid bad smell.
Necessity of post-treatment	Post-treatment of the anaerobic effluent is generally required to reach the discharge standards for organic matter, nutrients and pathogens.

However, in 1970s, the steep increase in energy prices reduced the attractiveness of aerobic processes and contributed to redirecting research efforts towards energy-saving alternatives like anaerobic treatment (van Haandel & Lettinga, 1994). In addition, the development of high rate anaerobic reactors has made it possible to achieve high levels of biomass in anaerobic reactors and long SRT while maintaining a short HRT. This has resulted in increased efficiency and utilization of anaerobic processes for the treatment of various wastewaters.

2.1.3 Anaerobic processes

The careful selection of the technology and appropriate reactor design and operation has overcome most of the possible difficulties of anaerobic treatment. Anaerobic processes can occur in suspended and attached-growth systems. In the past decades, the number of different anaerobic processes and the range of waste types that can or are being treated via anaerobic processes have been expanded. Among the processes are upflow anaerobic sludge blanket (UASB) (Lettinga *et al.,* 1980), anaerobic filter (AF) (Young & McCarty, 1969), anaerobic attached film expanded bed (AAFEB) (Jewell *et al.,* 1981), anaerobic fluidized bed reactor (AFBR) (Sanz & Fdz-Polanco, 1990; Collivignarelli *et al.,* 1991), packed-bed reactors (Collivignarelli *et al.,* 1991), anaerobic sequencing batch reactor (anSBR) (Dague *et al.,* 1966, 1970), and modified anaerobic baffled reactors (ABR) (Yu & Anderson, 1996).

2.1.3.1 Upflow anaerobic sludge blanket (UASB)

The UASB consists of a bottom layer of packed sludge, a sludge blanket and an upper liquid layer. Wastewater travels in an upward mode and biological degradation is achieved by contact with the microbes in the sludge bed. A settler screen located at the top of UASB separates the sludge flocs from the treated effluents and gas is collected through a gas outlet. The success of the UASB relies on the establishment of a dense sludge bed at the bottom of the reactor, in which the biological processes take place.

UASB reactor is a robust technology and has been used extensively for the treatment of several wastewaters. It is by far the most widely used high rate anaerobic process

for sewage treatment. Full-scale UASB reactors are now in operation in India, Colombia and Brazil (Sato *et al*., 2006; Wiegant, 2001). These UASB reactors are operated at HRT in the range of 5–19 h under ambient temperatures (18–32 ^{o}C) and organic loading rates (OLRs) in the range of 0.9–3.55 kg COD/m^{3}d. The removal efficiencies of tCOD, BOD and TSS achieved are in the range of 51–74%, 53–80%, and 46–80% respectively.

2.1.3.2 Anaerobic filter (AF)

An AF contains filter medium with a void space of approximately 50% or more. The bulk of anaerobic bacteria grow attached to the filter medium while some form flocs that become trapped inside the filter medium. The filter medium facilitates the retention of microbes in the reactor for a longer duration, hence achieving a longer SRT (Henze and Harremoes, 1983). Wastewater usually travels in an upward mode and biological degradation is achieved by contact with the microbes attached on the filter medium. The upflow of wastewater through the reactor also helps to retain suspended solids (SS) in the column.

However, the presence of SS in AF might cause clogging problem when wastewater passes through the media, leading to short circuiting of wastewater. This would lead to inadequate treatment of wastewater that in turn impair the reactor efficiency and yielding unacceptable effluent quality (Bodkhe, 2008). Hence for successful operation of AF for longer duration, the control of SS in the influent wastewater is essential (Jahren *et al*., 1999; Foresti, 2002).

The development of improved reactor configuration of AF to eliminate clogging problem was performed by Bodkhe (2008), treating municipal wastewater. It was found that a HRT of 12 hours was the most appropriate for the system studied. 90% BOD, 95% COD together with 95% SS reductions were achieved without any pretreatment. The specific biogas yield obtained was 0.35 m^3 CH_4 / kg COD with 70% of CH_4 content in the biogas generated.

Bodík *et al*. (2002) found that the average removal efficiency of COD for AF was 46 – 92% while experimenting on different temperature (8 – 20^oC) and HRT (6 – 20 hours) treating domestic wastewater.

2.1.3.3 Anaerobic sequencing batch reactor (anSBR)

A typical anSBR comprises of a cycle of 4 distinct stages: Feed, React, Settle and Decant. In the Feed stage, the wastewater is added to the reactor. Following the feed is the react stage where reaction and mixing takes place. During the Settle stage, mixing is stopped to allow for separation of biomass and solids from the liquid. Thus, the reactor acts as a clarifier to allow for gravitational settling. After the solids and liquids have been adequately separated, the Decant stage takes place. The supernatant is decanted and discharged from the reactor with minimum disturbance to the settled solids. Upon completion of the decant stage, the whole cycle repeats itself and the reactor is fed with a new batch of wastewater for the commencement of a new cycle.

anSBR has been successfully applied in laboratory and pilot scales for treatment of high strength wastewaters, such as landfill leachate (Kennedy and Lentz, 2000), swine

waste (Massé et al., 2003), brewery wastewater (Shao *et al*., 2007) and dairy wastewater (Dugba and Zhang, 1999).

anSBR could be applied successfully for low-strength wastewater too. Bodík *et al*. (2002) found that the average removal efficiency of COD for anSBR was 56 – 88% while experimenting on different temperature (8 – 20^{o}C) and HRT (6 – 20 hours) treating domestic wastewater.

A comparative study for the performance of three pilot-scale anSBR treating domestic sewage was conducted by Sarti *et al*. (2007). The three anSBR had different geometric characteristics (L/D ratio) and mixing type (mechanical mixer or liquor recirculation). It was found that mixing supplied by mechanical impeller was more stable and resulted in better organic matter removal efficiency, attaining 60% tCOD and 78% filtered COD removal. In addition, the average SS removal efficiency was 79%.

2.2 Post-Treatment

Anaerobic treatment has shown to be an efficient process for the removal of organic material and suspended solids from sewage, especially in regions with warm climate. However, it has little effect on the concentration of macronutrients (nitrogen and phosphorus) and pathogenic organisms. In order to meet the discharged standard, post-treatment is required to remove residual COD and SS and to reduce the concentrations of nutrients and pathogens.

Various systems have been proposed for post-treatment, such as submerged aerated biofilters (SAB) (Goncalves *et al.*, 1999), down-flow hanging sponge (DHS) (Tawfik *et al.*, 2006), rotating biological contactors (RBC) (Tawfik *et al.* 2002 and 2005), conventional activated sludge (CAS) (Huang *et al.* 2005), integrated duckweed and stabilization pond (Steen *et al.* 1999).

2.2.1 Rotating biological contactor (RBC)

The RBC process consists of a series of large discs with radial and concentric passages slowly rotating in a tank. About 40 percent of the media surface area is in the wastewater during the rotation. The rotation and subsequent exposure to oxygen allows organisms to multiply and form a thin layer of biomass on the disc that caused the biochemical degradation of organic pollutants. Excess biomass is sheared off at a steady rate and then carried through the RBC system for removal in a clarifier.

A single stage RBC system represented an efficient post-treatment process for a high quality anaerobically pre-treated domestic sewage (Tawfik *et al.*, 2002). Average residual effluent tCOD as low as 72mg/l can be obtained at an HRT of 2.5 hours and organic loading rate (OLR) of 10 g COD_{biod}/m^2.day. However, the performance of a two stage RBC system is better than that of a single stage RBC system in case of poor quality UASB reactor effluent. Moreover, the nitrification efficiency is higher than that of a single stage RBC system. Therefore, a two stage RBC system is recommended for a poor quality anaerobic effluent.

2.2.2 Down-flow hanging sponge (DHS)

The process of DHS is similar to the mechanism of the trickling filter, but uses polyurethane material (CF sponge) as the packed media for growth and attachment of active microorganisms. The CF sponge is also a porous media for solids retention. DHS could combine long sludge age with short HRT and provide small footprints for the bioreactors (Machdar *et al.*, 1997).

Tawfik *et al*. (2006) showed that a combined system consisted of UASB and DHS operated at a total HRT of 10.7 hours and a total SRT of 88 days represents a cost effective sewage treatment process. The average tCOD and $tBOD_5$ concentrations measured in the final effluent of the combined system amounted to 43 and 3.0 mg/l, respectively, corresponding to the overall removal efficiency of 90% for tCOD and 98% for $tBOD_5$. In addition, the combined system could produce a final effluent containing a low concentration of 12 mg/l TSS.

2.2.3 Conventional activated sludge (CAS)

CAS process consists of three basic components: (1) a reactor in which the microorganisms responsible for treatment are kept in suspension and aerated; (2) liquid-solids separation, usually in a sedimentation tank; and (3) a recycle system for returning solids removed from the liquid-solids separation unit back to the reactor. An important feature of CAS is the formation of flocculent settleable solids that can be removed by gravity settling in sedimentation tanks.

A study was conducted by Huang *et al*. (2005) using a combined UASB-CAS reactor system with consistently wasting of excess biomass to treat SS pre-settled piggery wastewater (COD = 2000mg/l, total Kjeldahl nitrogen (TKN) = 400mg/l, SS = 250-400mg/l). The results showed that the system could achieve efficient removal of COD (95-97%), TKN (100%) and TN (54-55%). It had shown that combined UASB-CAS system should be regarded a promising alternative for the removal of organic carbon and nitrogen from piggery wastewater.

2.2.4 Integrated duckweed and stabilization pond

The duckweed based stabilization pond functions as anaerobic pond except at the top layer where aerobic condition prevails. The top aerobic zone effectively controls the odour problems of the pond. The capability of up taking nutrients and other substrate from wastewater has attributed this plant to be biological purifier. There is remarkable reduction of BOD, COD, TSS, Nitrogen, Phosphorus and Heavy metals from wastewater in duckweed based stabilization pond.

A study using integrated pond system for post-treatment of effluent from an UASB reactor, which was fed with domestic sewage was conducted by Steen *et al*. (1999). The system consisted of a series of shallow duckweed and stabilization ponds with an overall retention time of 4.2 days. Rapid and efficient pathogen removal was achieved in shallow stabilization ponds but the effluent BOD and TSS was relatively high, due to presence of algae. Effluent quality of 11mg/l TSS was attainable when practically all algae were removed by passing stabilization pond effluent through a stage with reduced solar radiation.

2.3 Membrane bioreactor (MBR)

A MBR comprises of two components: a bioreactor and a membrane module. The biological component is responsible for the biochemical degradation of waste compounds while the membrane module accomplishes the solids-liquids separation.

The MBR process can produce high quality effluent with high BOD_5 removal (about 98%), complete nitrification and partial denitrification (Kishino *et al*, 1996; Fan *et al*., 1996; Cicek *et al*., 1998). Virtually complete TSS removal is achievable with low turbidity values of less than 0.3 NTU and MBR effluents can be used for reverse osmosis feed with moderate success (Lozier *et al*., 1999). In addition, the dependence on disinfection is also reduced due to the trapping of a significant proportion of pathogenic organism by the membrane.

2.3.1 Configuration of MBR

Presently, there are two main MBR configurations, namely the submerged MBR and the side-stream MBR. The transmembrane pressure (TMP) is derived either from the hydraulic head of the water above the membrane, or by the use of a suction pump.

2.3.1.1 Submerged MBR

For the submerged type, membrane modules are directly immersed in the activated sludge tank. Aeration is supplied underneath the membrane modules, generating

sufficient oxygen for biological reactions. This also ensures complete mixing in the bioreactor and reduces fouling by scouring the membrane with air bubbles. Simultaneous biological nutrient removal can be achieved by incorporating anoxic or anaerobic compartments in the system. The membrane is cleaned by periodic permeate back-pulsing or chemical backwashing. Figure 2 shows the schematic diagram of a submerged MBR.

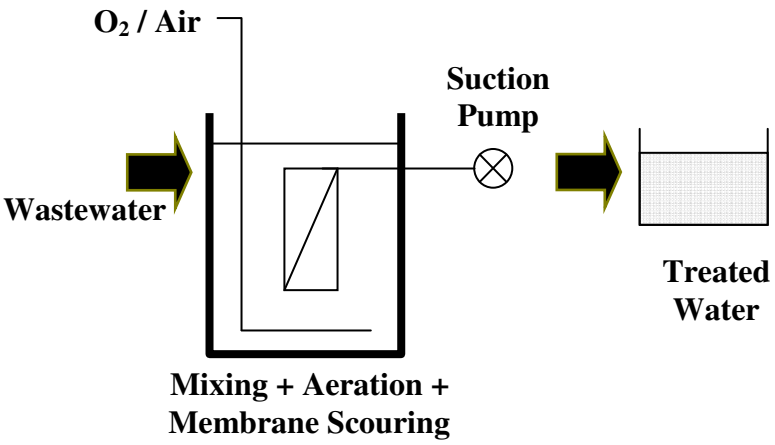


Figure 2.2 Schematic diagram of a submerged MBR (Stephenson *et al.*, 2000)

2.3.1.2 Side-stream MBR

For the side-stream MBR, membrane filtration is achieved outside of the activated sludge tank. The activated sludge is pumped from the bioreactor to a pressure-driven membrane module where solids retain behind the membrane while the permeate passes through it. The cross-flow is normally generated by a pump that provides pressure for the membrane filtration process. The retained sludge is returned to the bioreactor. Figure 3 shows the schematic diagram of a side-stream MBR.

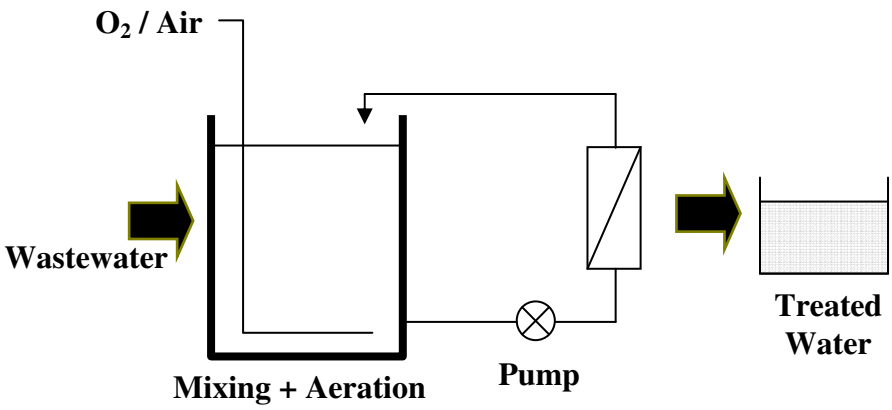


Figure 2.3 Schematic diagram of a side-stream MBR (Stephenson *et al.*, 2000)

As such, these two configurations can be distinguished by the technology used to create pressure gradient between the two sides of the membrane. The pressure across the submerged MBR is applied by suction through the membrane or by pressurizing the reactor. On the other hand, pressurizing the flow through the membrane creates pressure gradient in the side-stream MBR. Due to the emergence of less costly membranes with lower pressure requirements and higher permeate fluxes; there has been a strong trend towards the usage of submerged MBR.

2.3.2 Factors affecting MBR process

Although MBR offers many advantages over CAS, membrane fouling remains a major drawback. Fouling leads to significant increase in hydraulic resistance. It causes permeate flux decline or TMP increase when the process is operated under constant-TMP or constant-flux conditions, respectively. Thus, it results in a higher energy usage, a higher cleaning frequency and a shorter life span of the membrane.

The membrane fouling is dependent on various parameters concerning the membrane characteristics, the operational conditions and the activated sludge characteristics. Among these are aeration rate, critical flux and SRT.

2.3.2.1 Aeration rate

In a submerged MBR, in addition to providing oxygen to biomass and keeping solids in suspension; aeration is also used to scour the membrane surface to minimize membrane fouling. Coarse bubble diffuser is generally used in submerged MBR. The rising bubbles will provide a turbulent crossflow velocity (approximately 1 m/s) over the surface of the membrane. It helps to maintain flux through the membrane by reducing the build up of material at the membrane surface and thereby increases the operational cycle of the system.

A high aeration rate certainly can reduce biomass attachment to the membrane; however Han *et al*. (2005) showed that the cake-removing efficiency of aeration did not increase proportionally with the increase in the air flow rate and there was an optimum value for the cake-removing.

Meng *et al*. (2007) showed that either small or large aeration intensity had a negative influence on membrane permeability. The large aeration intensity would result in severe breakup of sludge flocs and promote the release of colloids and solutes from the microbial flocs to the bulk solution. Under an aeration intensity of 150 l/h, Brownian diffusion was the main back transport mechanism for membrane foulants, which could

not remove the cake layer effectively. The cake resistance under an aeration intensity of 150 l/h was more than two times of that of 400 l/h and 800 l/h, thus indicating that aeration has great impacts on the removal of cake layer.

2.3.2.2 Critical flux and SRT

The concept of critical flux was originally presented by Field *et al*. (1995). It is defined as the maximum flux at which the membrane system can operate without accumulation of foulants. Alternatively, a stable filtration operation with constant permeability for an extended period of time has been defined as sub-critical operation even when preceded by an initial decline in flux due to solute absorption (Howell, 1995). The sub-critical operation is expected to lead to little or even no irreversible fouling. However, recent observations showed that fouling can take place in a MBR even under the critical flux (Le-Clech et al., 2003), but the fouling rate is at a much more sustainable level.

Due to the complex mechanisms underlying membrane fouling, Fane *et al*. (2002) pointed out that the critical flux could be affected by three groups of factors including membrane materials and configurations, operating parameters and sludge characteristics.

SRT can produce significant effects on biomass properties in a MBR. With perfect solid-liquid separation providedby the membrane, MBR can maintain high MLSS and SRT. A higher biomass concentration would give rise to higher treatment efficiency.

However, higher MLSS concentrations can accelerate membrane fouling via rapid deposition of sludge particles on the membrane surface (Takeshi and Yasuhiko, 1991). In addition, it was reported that mixed liquor properties such as viscosity, amount and composition of microbial produce and cell surface properties were changed at longer SRT (Shin and Kang, 2003; Chang and Lee, 1998), which will also influence membrane fouling.

Numerous studies on the effects of MLSS concentration on critical flux or filterability had been carried out. Madaeni et al. (1999) observed that critical flux was inversely related to MLSS concentration from 0 to 10 g/l. However, Rosenberger and Kraume (2002) showed that MLSS concentrations between 2 and 24 g/l had little influence on filterability. Another study by Le-Clech *et al*. (2003) indicated that there was little difference in critical flux for MLSS concentration ranging from 4 to 8 g/l, but there was a significant increase in critical flux when MLSS concentration was increased to 12 g/l.

Additional research had shown that small colloidal particles of around 1 mm in diameter might play a critical role in membrane fouling in MBR systems (Chang and Kim, 2005). EPS was identified as the most significant biological factor (Chang et al., 2002). Consistently, Fan *et al*. (2006) found that the critical flux can be correlated to soluble EPS while being independent of bound EPS.

2.4 Nitrogen removal

In anaerobic treatment, the nitrogen concentrations are largely unaffected while the COD concentration is reduced; therefore the ratio of COD/N ratio tends to decrease.

Biological nitrogen removal is performed through two individual sequential processes: nitrification and denitrification. This is the most commonly used process for nitrogen removal from domestic and industrial wastewater.

During nitrification, ammonia is oxidized to nitrate with nitrite as an intermediary compound, by the action of autotrophic nitrifying bacteria that use ammonia (and nitrite) as energy source. During denitrification, nitrate is reduced to gaseous nitrogen (with nitrite, nitrous oxide and nitric oxide as intermediaries) by the action of anoxic bacteria that use NO_x as final electron acceptor. Organic matter is the electron donor for this process.

In an aerobic wastewater treatment system, aerobic heterotrophs, strict aerobic nitrifiers and facultative aerobic denitrifiers often exist together. The populations of the three different types of microorganisms vary with different wastewater characteristics and operating conditions. They would compete with one another for substrates in the wastewater and eventually maintain a delicate stable relationship. Several single sludge processes such as A/O, A2/O, SBR and VIP were developed (Metcalf & Eddy, 2003) with different combinations of anaerobic, anoxic and aerobic zones or compartments employed in the activated-sludge process.

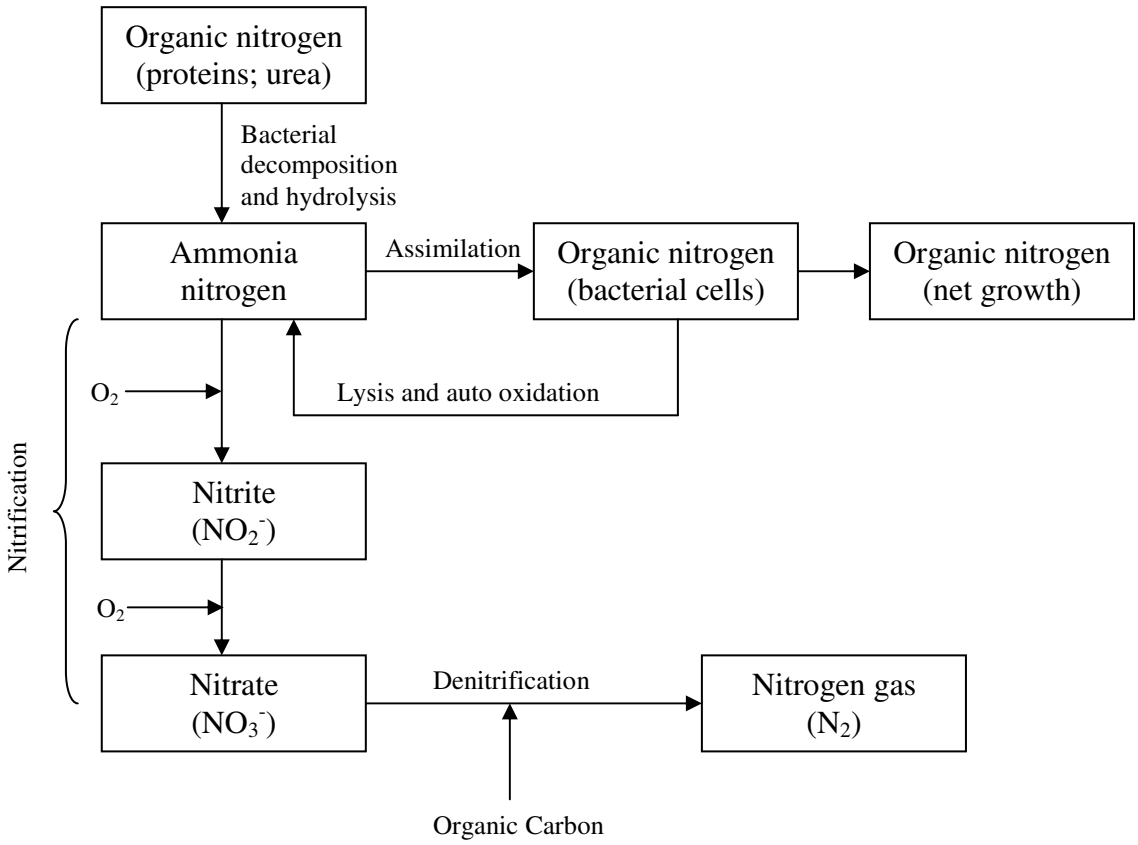


Figure 2.4 Nitrogen transformations in biological treatment processes. (Adapted from Sedlak, 1991)

To achieve a good nitrogen removal performance, favourable operating condition is essential in order to maintain delicate stable relationship between the three different microorganisms.

2.4.1 Biological nitrification

Biological nitrification is a two step process in which ammonia (NH_4^+-N) is oxidized to nitrate (NO_3^--N) via nitrite (NO_2^--N) by two distinctly different autotrophic bacteria, *Nitrosomonas* and *Nitrobacter*. The overall stoichiometric reactions in the oxidation of ammonia to nitrate are as followed (Tchobanoglous and Burton, 2004):

Nitroso-bacteria: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$ (2.2)

Nitro-bacteria: $2NO_2^- + O_2 \rightarrow 2NO_3^-$ (2.3)

Total oxidation reaction: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + 2H_2O$ (2.4)

Based on the total oxidation reaction, the oxygen required for complete oxidation of ammonia is 4.57 g O_2/g N oxidized. 3.43 g O_2 is used for nitrite production and 1.14 g O_2 is used to oxidize nitrite.

Various parameters influence the nitrification process. These parameters include dissolved oxygen (DO), temperature, pH, organic loading and sludge retention time (SRT).

2.4.1.1 DO concentration

DO is the key limiting factor for nitrification process. It was reported that low a DO concentration can inhibit nitrification process of activated sludge. The stoichiometric quantities of oxygen required according to Equation (2.4) are: 3.43mg for nitrification of 1mg NH_4-N and 1.14mg for nitratification of 1mg NO_2-N. The theoretical nitrogeneous oxygen demand (NOD) is 4.57mg per mg of NH_4-N.

Rittmann and McCarty (2001) reported that continued operation with a DO level below K_{O_2} (K_{O_2} of ammonia-oxidizing bacteria = 0.5 mg O_2/l; K_{O_2} of nitrite-oxidizing bacteria = 0.68 mg O_2/l) will lead to biomass washout and high NH_4^+-N concentration in the effluent. Under low DO level condition, NH_4^+-N is unable to be fully oxidized

to NO_3^--N, thus will stop as the intermittent product NO_2^--N. Ruiz *et al.* (2006) found that nitrite accumulation took place at DO concentration of between 0.7 and 1.5 mg/l; and ammonia oxidation was affected at a DO concentration of 0.5 mg/l. Thus a minimum DO concentration of 2.0 mg/l is usually recommended for complete nitrification performance to be achieved in a treatment process (Tchobanoglous and Burton, 2004; Eckenfelder and Grau, 1992).

In contrary, Hanaki *et al.* (1990) showed that low DO (0.5mg/l) did not affect ammonia oxidation as a whole in a pure nitrification system; however nitrite oxidation was strongly inhibited. Thus a minimum DO concentration of 2.0 mg/L is usually recommended for complete nitrification performance to be achieved in a treatment process (Tchobanoglous and Burton, 1991; Eckenfelder and Grau, 1992).

The inhibitory effect of organic loading on ammonia oxidation was enhanced by low DO (Hanaki *et al.*, 1990). The impact becomes more significant when fast-growing heterotrophic bacteria compete with autotrophic nitrifying bacteria for the limited oxygen as the organic loading in the reactor increases. Grady and Lim (1980) had reported that heterotrophic bacteria have a maximum growth rate of five times and yields of two to three times than that of autotrophic nitrifying bacteria.

2.4.1.2 Temperature and sludge retention time (SRT)

The activity of the nitrifying bacteria in activated sludge mainly depends on temperature and SRT. Randall *et al.* (1992) observed that in the aerated reactor near complete nitrification was accomplished at 20^{o}C with SRT of 2.7 days. When the

temperature decreased to 10^{o}C, even with SRT of 5 days, the nitrification effectiveness was lower than 65%. The effect of temperature in the range of 10 – 20^{o}C was not observed when SRT was 15 days. The increase of temperature by 1^{o}C would result in about 10% reduction of SRT required for nitrification (Sinkjær *et al*., 1994). Generally, the SRT above 20 days eliminate unfavorable influence of low temperatures and stabilize the nitrification process.

2.4.1.3 Organic loading - COD/N ratio

Presence of organic matter will provoke the growth of heterotroph, which assimilate the ammonia and reduce availability of ammonia for nitrifying bacteria (Hanaki *et. al*. 1990). Hence, the limitation of organic loads in influent to aeration zone of the reactor is advisable to reduce competition between oxidation and nitrification.

A comparative study conducted by J. Akunna *et. al*. (1994) showed that 3% of added ammonia nitrogen was used by autotrophic nitrifiers for cell synthesis during nitrification of the autotrophic medium, while up to 30% was used for both autotrophic and heterotrophic cell synthesis during oxidation and nitrification of the anaerobically pre-treated effluent. In addition, heterotrophic growth could completely inhibit nitrification in an aerobic filter even in the presence of sufficient dissolved oxygen and abundant ammonia nitrogen.

The nitrification process was stable and could achieve more than 95% effectiveness when COD/N ration was lower than 4 (Komorrowska-Kaufman *et al.,* 2006). In contrast, Ling and Chen (2005) found that the reduction of nitrification rates was

about 60-70% for a substrate concentration of 10 mg NH_4^+-N/l when the COD/N ratio increased from 0 to 3.

2.4.1.4 pH and alkalinity

Nitrification is pH-sensitive and operates at a narrow optimal range. Tchobanoglous and Burton (2004) reported that the optimal range lies between 7.5 and 8.6 while USEPA (1975) suggested that nitrification rate can be assumed to be constant at pH between 7.2 and 8.0.

Grunditz and Dalhammar (2001) found that the optimal pH for *Nitrosomonas* and *Nitrobacter* are 8.1 and 7.9, respectively, based on pure culture isolated from activated sludge. Another study by Boller *et al.* (1994) showed that nitrification rate in a biofilm reactor declined rapidly when pH is below 7.0 and ceased when pH is within the range of 6.5 to 6.7.

Based on Equation (2.2), H^+ ions are produced during ammonia oxidization. The H^+ ions will react with alkalinity (OH^-, HCO_3^-, CO_3^{2-}) available in the wastewater. According to the stoichiometry of the biochemical reaction, 1g NH_4^+-N will consume 7.14g of alkalinity. If insufficient alkalinity is available in the wastewater, pH dropped to 6.2 would result in cessation of nitrification in activated sludge (Painter, 1970). Thus, addition of lime or soda ash may be required to maintain pH at an optimal level.

The effect of pH on nitrification can be linked to the availability of carbon source and the existence of unionized ammonia and nitrous acid. In a carbonate system ($CO_2 \Leftrightarrow$

$HCO_3^- \Leftrightarrow CO_3^{2-}$) such as the mixed liquor, high pH would transform the mineral carbon to insoluble carbonate which is hardly assimilable to the nitrifers. For low pH, the predominated CO_2 can be stripped from the mixed liquor by the aeration which would result in alkalinity scarcity (Villaverde *et al.*, 1997).

2.4.2 Biological denitrification

Biological denitrification involves the biochemical oxidation of organic substrates in wastewater using nitrate or nitrite as the electron acceptor instead of oxygen. It is carried out in the absence of DO or under limited DO concentrations. Denitrification involves the following reduction steps from nitrate to nitrite, nitric oxide, nitrous oxide and nitrogen gas:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (2.5)$$

The stoichiometric reactions for different electron donors are as followed (Tchobanoglous and Burton, 2004):

Wastewater:

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \quad (2.6)$$

Methanol:

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad (2.7)$$

Acetate:

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \quad (2.8)$$

Unlike nitrification which required a specialized group of bacteria, a wide range of heterotrophic and autotrophic bacteria are capable of denitrification.

Denitrification has been reported to be able to perform by chemoorganotrophic, lithoautotrophic, and phototrophic bacteria and some fungi (Bothe *et al.*, 2000). Denitrifiers are common among the Gram-negative α– and β–proteobacteria such as *Pseudomonas, Alcaligenes, Paracoccus,* and *Thiobacillus*. Gram-positive bacteria such as *Bacillus* and a few halophilic Archaea such as *Halobacterium* are also able to denitrify (Rittmann and McCarty, 2001). For wastewater treatment, denitrification is mainly carried out by a group of diverse facultative aerobes that respire nitrite or nitrate under oxygen reduce or anoxic condition; and with organic compounds as the carbon and energy source.

2.4.2.1 Organic loading - COD/N ratio

Denitrification intensity depends on carbon availability. In order to denitrify all nitrates arisen in the nitrification process, the carbon to nitrogen (C/N) ratio in the influent should be high enough. In the case that sufficient carbon is unavailable, delivery of readily biodegradable carbon directly to anoxic zone should be provided.

Using acetic acid as an external carbon source, the chemical equilibrium equation including cell synthesis has been suggested by Mateju *et al.* (1992) as:

$$0.819CH_3COOH + NO_3^- \rightarrow 0.068C_5H_7NO_2 + HCO_3^- + 0.301CO_2 + 0.902H_20 + 0.466N_2 \quad (2.9)$$

Oxidation of acetic acid

$$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O \quad (2.10)$$

Based on Equation (2.9), the reduction of 1g NO_3^--N will consume 3.51g acetate (or 2.74g COD) to produce 0.55g new cells, 2.66g alkalinity (expressed as HCO_3^-) and 0.82 l inert nitrogen gas. The theoretical optimal COD/N is calculated to be 3.74, without competition from other heterotrophs.

Based on the transfer of one electron equivalent for dissimilative reduction of nitrate (i.e. denitrification), the reduction of 1g of NO_3^--N requires the consumption of 2.86g of COD.

The theoretical C/N determined by Henze *et al*. (1994) was 3.5–4.5 g ΔCOD / g ΔN, however higher C/N requirements of 6-11 g ΔCOD / g ΔN was proposed for single sludge system with pre-denitrification. For full-scale pre-denitrification system, Water Polution Control Federation (1983) recommended that the influent COD/N ratio should be higher than 15. The reason for the excessive COD requirement in the pre-denitrification mode was caused by rapid initial COD removal via absorption. For complete denitrification, the required COD/N ratio reported by Kujawa and Klapwijk (1996) was equal to 8.4 when organic source comes from wastewater while the COD/N ratio for acetic acid was in the range of 4.0 to 9.2.

In general, the required COD/N ratio would depend on the biological denitrification process and the specific wastewater; and the optimal COD/N ratio can only be determined experimentally (Chiu and Chung, 2003).

2.4.2.2 pH and alkalinity

Alkalinity is released during denitrification in the single-sludge reactor system. According to the stoichiometry of the biochemical reaction as shown in Equation (2.9), 1 mole of HCO_3^- is generated for every mole of NO_3^- being denitrified to N_2. 1g of NO_3^- will release 3.57g of alkalinity. In contrast to nitrification, there is less concern about pH influence on denitrification rate. No significant effect on the denitrification rate is reported for pH between 7.0 and 8.0, however Dawson and Murphy (1972) showed that there was a decrease in denitrification rate as the pH decreased from 7.0 to 6.0 in batch acclimated tests.

In general, denitrifiers are less sensitive to pH and can carry out denitrification over a wide range of pH conditions ranging from 6 to 8 (WEF and EWRI/ASCE, 2006). Drtil *et al.* (1995) reported that denitrification would be inhibited when pH exceeded 8.3. Although pH condition would not limit the nitrogen removal performance of denitrification, it would affect the type of end product produces from the process. Improper control of pH condition would result in the formation of the undesirable nitrous oxide (N_2O) that will act as a powerful greenhouse gas instead of dinitrogen (N_2). Denitrification at pH above 7.3 would give rise to N_2 production while pH below 7.3 would give rise to N_2O production (Delwiche, 1970). Similar finding by Hanaki *et*

al. (1992) also showed that N_2O production at pH 6.5 was significantly higher than that at pH 7.5. Thus pH condition would not affect the nitrogen removal performance of denitrification process but it would inhibit the formation of N_2 from N_2O.

2.4.2.3 Mixed liquor recycle ratio

For a pre-denitrification system, the internal mixed liquor recycle flow from the oxic to the anoxic zones plays an important role in TN removal efficiency. A high mixed liquor recycle flow will bring more nitrates back to anoxic zone for denitrification, hence reducing the nitrates that escape with the effluent.

Jih *et al*. (2001) observed that increasing mixed liquor recycle ratio from 1 to 3 only imposed a slight effect on TN removal efficiency. Van Haandel et al. (1981) pointed out that denitrification rate should have reached a maximum value if the oxidized forms of nitrogen were detected in the effluent from the anoxic reactor. Therefore, the denitrification rate would not increase with a further increase in mixed liquor recycle ratio.

The aeration condition, which affects the DO concentration in the mixed liquor, in the MBR is very much different from that of the CAS. High aeration rate is usually used in MBR to control membrane fouling (Liu *et al*., 2000; Germain et al., 2005), hence DO concentration in the MBR can easily be above 4 mg O_2/l (Chu and Li, 2005; Yoon *et al*., 2004). A high mixed liquor recycle flow, typically required for effective denitrification, will also bring a large amount of DO from the oxic zone to anoxic zone. As denitrifiers are facultative bacteria that energetically prefer oxygen than nitrate as

the terminal electron acceptor, a high DO present in high mixed liquor recycle flow would inevitably deteriorate the TN removal efficiency.

A study on conflicting influence of mixed liquor recycle ratio and dissolved oxygen on nitrogen removal and membrane fouling of a pre-denitrification submerged MBR was conducted by Tan and Ng (2007). It was found that a high DO concentration (average of 5.1±0.5mg O_2/L) present in the recycle mixed liquor at an aeration rate of 10 l air/min deteriorated the TN removal efficiency when operating at a recycle ratio of more than 3. However, a lower aeration rate of 5 l air/min, resulted in an average DO concentration of 3.4±0.7mg O_2/l in the recycle mixed liquor, led to an improvement in TN removal efficiency: 63%, 80%, 84% and 89% for mixed liquor recycle ratio of 1, 3, 5 and 10, respectively. Further decrease in aeration rate to 2.5 l air/min, resulting in an average DO concentration of 1.9±0.8mg O_2/l, did not improve the TN removal efficiency.

CHAPTER THREE MATERIALS AND METHODS

3.1 Experimental setup

Three CAS and three MBR were set up for post-treatment of UASB, AF and anSBR effluents. The study was divided into two phases. Phase 1 was designed to study the performance of post-treatment for organic removal and nitrification under two different HRTs, while phase 2 focused on the effect of influent COD concentration for the performance of denitrification by varying the ratio of anaerobic effluent and domestic sewage. Table 3.1 summarizes the two phases for this study.

Table 3.1 Summary of the phases of the study

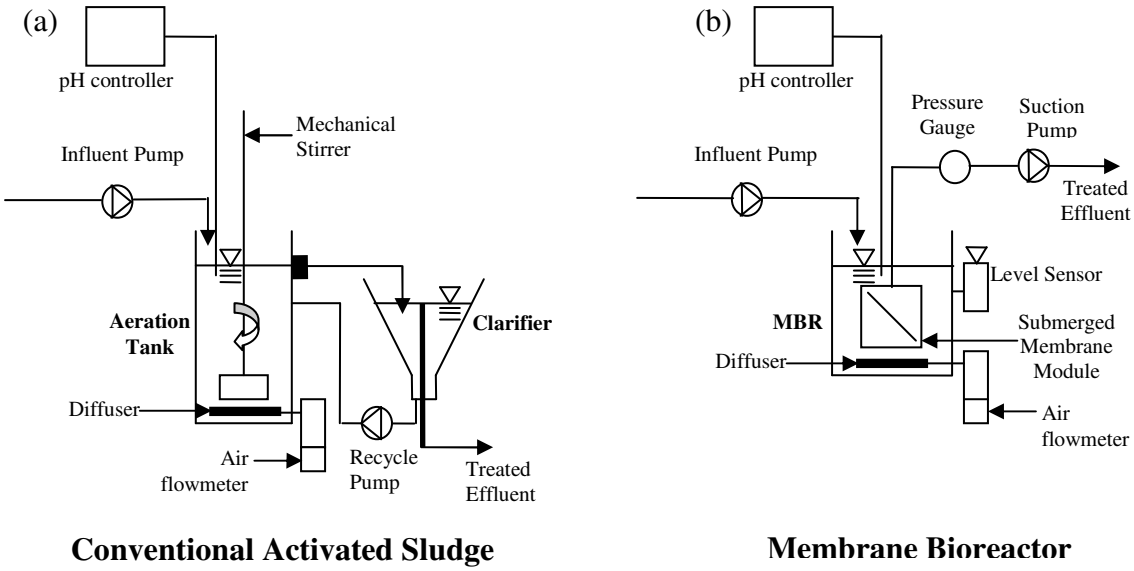
Phase 1 - Nitrification Study
16 hr HRT pre-treatment + 8 hr HRT post-treatment
6 hr HRT pre-treatment + 4 hr HRT post-treatment
Phase 2 - Denitrification Study*
100% anaerobic effluent
25 % domestic sewage + 75 % anaerobic effluent
50 % domestic sewage + 50 % anaerobic effluent

* All 3 anaerobic pre-treatment systems were operated at 6 hr HRT, while post-treatments for UASB, AF and anSBR were operated at 6, 8 and 6 hr HRT, respectively

3.1.1 Phase 1 – Nitrification study

During the study of nitrification performance, CAS consisted of an aeration tank and a clarifier, while MBR consisted of an aeration tank with submerged membranes. Working volume for both aeration tanks was 4.5L. Settled sludge from CAS clarifier was returned back to aeration tank at 1 time influent flow rate (1Q) to maintain sufficient sludge concentration.

Both systems were fed continuously with effluent from the three anaerobic pre-treatment individually through variable speed pumps (Cole Parmer caustic pump controller and Masterflex peristaltic caustic pump). They were operated simultaneously, under similar conditions, but for different SRT of 10 and 20 days for CAS and MBR, respectively. Excess activated sludge was discharged daily from the aeration tanks through a sampling port to maintain the desired SRT. The schematic diagrams of the treatment process set up for nitrification study are illustrated in Figure 3.1.



Conventional Activated Sludge **Membrane Bioreactor**

Figure 3.1 Schematic diagrams of (a) Conventional Activated Sludge (CAS) and (b) Membrane Bioreactor (MBR) system for nitrification study

3.1.2 Phase 2 – Denitrification study

4.5L anoxic tanks were added to both CAS and MBR when study of denitrification began. Preanoxic denitrification was applied in this study. A top mounted stirrer was used in the anoxic tank to keep the mixed liquor inside the bioreactor homogeneous. Nitrite and nitrate produced in the aeration tanks were recycled back to the anoxic tanks. The recycle rate for CAS was 2Q from the aeration tank and 1Q from the clarifier, while recycle rate was maintained at 3Q from aeration tank for MBR.

Both systems were fed continuously with different ratio of anaerobic effluent and raw sewage through variable speed pumps (Cole Parmer caustic pump controller and Masterflex peristaltic caustic pump). The SRTs were 20 days and 40 days for CAS and MBR, respectively. The schematic diagrams of treatment process set up for denitrification study are illustrated in Figures 3.2 and 3.3.

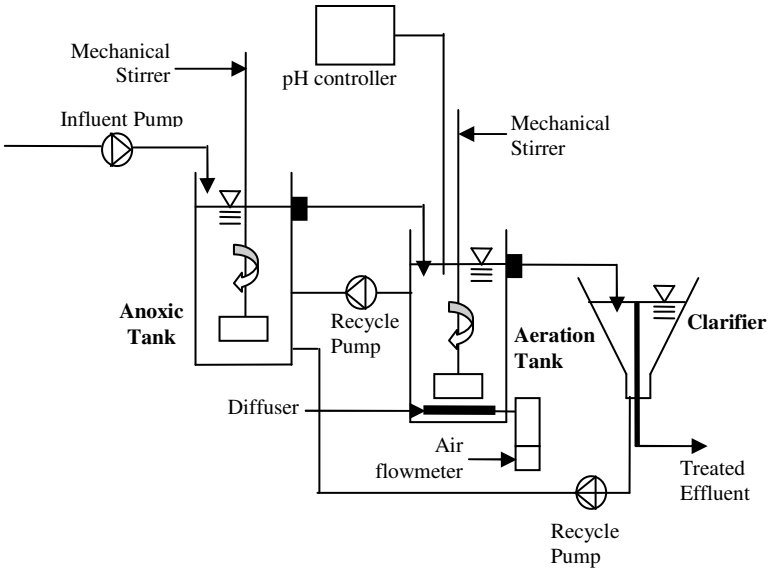


Figure 3.2 Schematic diagram of Conventional Activated Sludge (CAS) system for nitrification and denitrification study

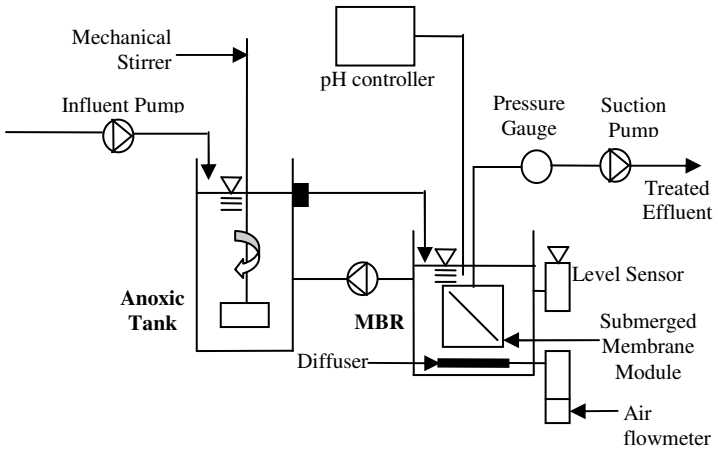


Figure 3.3 Schematic diagram of Membrane Bioreactor (MBR) system for nitrification and denitrification study

3.2 Operating conditions

All systems were operated at ambient temperature (25^{o}C to 32^{o}C) and with pH at between 6.8 and 7.5. The pH was maintained by a pH controller (αlpha-pH800, pH/ORP controller, EUTECH Instruments) using 0.2N sodium carbonate as the buffering agent and measured with an epoxy-body pH electrode (Cole-Parmer Instrument Co.) placed in the aeration tanks of both CAS and MBR.

Compressed air was controlled by air flow meter (Aalborg Instruments & Controls, Inc., USA) and supplied through diffusers located at the base of aeration tanks for both CAS and MBR. This is to ensure complete mixing of the biomass within the aeration tanks and also to serve as a mean to maintain dissolved oxygen (DO) level to be above 2.0 mg/L.

The membrane module used was Sterapore-L hollow fibre membrane from Mitsubishi Rayon Co. Table 3.2 summarizes the membrane specification used in this study. Nine membrane modules were used in parallel at all operating conditions. In order to minimise cake formation on the membrane surface, the membranes were operated intermittently (8 min on, 2 min off). In addition, the aerator placed below and in-line with each membrane module provided coarse bubbles to agitate the membrane fibers.

Table 3.2 Membrane specification

Membrane	
Membrane material	Polyethelene hollow fiber (hydrophilic)
Pore size	0.4 μm
Effective surface area	0.03 m^2
Suction cycle	10 min (8 min on; 2 min off)
pH	2 – 11
Operating pressure	< 25 kPa
Operating temperature	< 40^oC

3.3 Reactor start-up and operation

3.3.1 Inoculation

The aeration tanks of both CAS and MBR were started up on Day 0 using seed sludge collected from the activated sludge tank at Ulu Pandan Water Reclamation Plant (UPWRP). Both systems were inoculated up to a concentration of approximately 2000 mgVSS/L.

To ensure similar microbial community, anoxic tanks were seeded with activated sludge collected from the respective aeration tanks. Anaerobic effluent and post-treatment effluent were added to achieve the desire volume. The ratio of anaerobic effluent, post-treatment effluent and activated sludge was 2:2:1. Concentrations of inoculation for anoxic tanks were approximately 3000 and 4000 mgVSS/L for CAS and MBR, respectively.

3.3.2 Domestic sewage

Domestic sewage was collected from UPWRP twice weekly. The collected domestic wastewater was added into a common holding tank after passing through a 2 mm pore size sieve daily and the temperature of the domestic wastewater was maintained at 30^{o}C using an electric heater. The domestic wastewater holding tank was equipped with a top mount stirrer to keep the influent wastewater homogeneous. Unused domestic wastewater was stored in the cold room at 4^{o}C for preservation.

Post-treatments for UASB and AF were fed from a common feeding tank while post-treatment for anSBR was fed from a separate tank. It was found that the characteristics of the domestic sewage fluctuated between different batches of sample. Table 3.3 summarizes the characteristics of the sieved domestic sewage.

Table 3.3 Characteristics of the sieved domestic sewage

Parameter	Concentration (mg/L)	
	UASB / AF	anSBR
NH_4^+-N	38.4 ± 6.7	35.5 ± 8.6
NO_2^--N	N.D.	N.D.
NO_3^--N	N.D.	0.1 ± 0.1
TN	53.6 ± 12.9	51.8 ± 14.3
DN	29.5 ± 9.8	30.5 ± 9.2
tCOD	544.1 ± 185.3	430.3 ± 225.6
sCOD	81.3 ± 34.3	86.9 ± 34.2
tBOD	199.0 ± 64.5	92.6 ± 41.7
sBOD	24.3 ± 11.4	19.7 ± 8.4
TOC	44.2 ± 18.7	43.3 ± 15.4
DOC	21.6 ± 7.4	20.2 ± 5.6
TSS	458.6 ± 224.3	361.1 ± 130.9
VSS	323.3 ± 156.3	277.3 ± 104.3

3.3.3 Anaerobic effluents

Domestic sewage collected from UPWRP was pre-treated with 3 different anaerobic systems (UASB, AF and anSBR). Anaerobic effluents from the 3 systems were then fed to CAS and MBR for post-treatment. Two different HRTs of 16 and 6 hours were operated for anaerobic systems during phase 1 of the study. Tables 3.4 and 3.5 summarize the characteristics of anaerobic effluents from the 3 systems operating at 16 and 6 hours HRT, respectively.

Table 3.4 Characteristics of anaerobic effluents at 16 h HRT

Parameter	Concentration (mg/L)		
	UASB	AF	anSBR
NH_4^+-N	39.6 ± 3.4	37.7 ± 3.9	35.8 ± 5.1
NO_2^--N	N.D.	N.D.	N.D.
NO_3^--N	0.2 ± 0.3	0.2 ± 0.3	0.2 ± 0.4
TN	36.5 ± 9.2	39.6 ± 8.7	37.6 ± 8.0
DN	30.2 ± 3.4	29.1 ± 3.4	27.5 ± 3.5
tCOD	227.9 ± 201.4	239.5 ± 195.8	229.4 ± 181.1
sCOD	49.7 ± 17.7	42.8 ± 22.9	48.1 ± 21.0
tBOD	23.0 ± 2.2	53.3 ± 4.8	61.9 ± 3.9
sBOD	8.8 ± 1.0	6.7 ± 0.8	9.0 ± 2.1
TOC	16.6 ± 6.7	19.8 ± 10.2	19.3 ± 5.7
DOC	10.4 ± 1.8	10.4 ± 1.8	10.6 ± 1.9
TSS	141.1 ± 184.4	219.5 ± 177.6	177.4 ± 129.1
VSS	102.3 ± 126.4	147.9 ± 119.9	128.6 ± 97.8

Table 3.5 Characteristics of anaerobic effluents at 6 hr HRT

Parameter	Concentration (mg/L)		
	UASB	AF	anSBR
NH_4^+-N	41.2 ± 7.7	37.9 ± 5.7	39.4 ± 6.4
NO_2^--N	N.D.	N.D.	N.D.
NO_3^--N	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.1
TN	47.6 ± 10.7	47.8 ± 12.5	47.3 ± 13.1
DN	32.0 ± 8.4	31.8 ± 5.4	32.2 ± 6.8
tCOD	352.6 ± 178.3	432.9 ± 201.8	306.6 ± 161.1
sCOD	58.4 ± 17.4	70.6 ± 18.9	53.2 ± 22.3
tBOD	89.1 ± 53.5	137.6 ± 54.3	79.9 ± 53.5
sBOD	10.3 ± 3.1	17.7 ± 6.9	9.5 ± 5.1
TOC	31.8 ± 16.0	35.4 ± 12.1	26.7 ± 11.0
DOC	14.1 ± 3.4	17.9 ± 4.0	15.5 ± 9.0
TSS	294.4 ± 196.7	325.2 ± 188.4	251.6 ± 179.8
VSS	208.1 ± 141.7	253.8 ± 146.1	186.5 ± 126.1

3.4 Sampling methods

3.4.1 Liquid samples

Feed and effluent samples were collected from their respectively inlet and outlet tubing. Mixed liquor samples were collected from the sampling port located at mid height of each zone. Sampling always started from the effluent before proceeding upstream to the feed. This is to ensure fewer disturbances to the upstream process during sample collection at the downstream.

To obtain the supernatant from the mixed liquor and soluble portion of the feed and CAS effluent, the collected samples were centrifuged at 4°C for 10 minutes at 9,000 rpm before undergoing filtration through a 0.45µm filter (Membrane Filter: GN-6 grid 47mm, 0.45µm, Pall Corporation, USA). Unused samples for future analysis were stored immediately in 4°C cold room and analyzed within a week.

3.4.2 EPS extraction

Extracellular polymeric substance (EPS) was separated from the microorganism cell wall by using cation resin exchange. Cation exchange resin (CER) will remove cations from the sludge matrix leading to a break up of the flocs and a subsequent release of EPS. The CER was firstly washed in phosphate buffer and stirred for an hour. Thereafter, the CER was kept while the phosphate buffer was decanted. 75mL of the sludge sample was centrifuged for 10 minutes at 9,000 rpm (4°C). The supernatant was decanted and resuspended to the original volume using phosphate buffer. 70g CER/g VSS was then added to the suspension in an open-mouth closed container. The suspension was stirred at 600 rpm for 1.5 hours in the cold room (4°C). Next, the suspension was centrifuged at 9,000 rpm for 10 minutes to separate the CER and biomass. The supernatant was collected for subsequent analysis of EPS.

3.4.3 DNA extraction

The mixed liquor genomic DNA was extracted using the chemical extraction method. Cells from the mixed liquor were collected from the aeration and anoxic tanks and immediately prepared for DNA extraction. The cells were first incubated with the

extraction buffer (Tris-HCl, EDTA and sucrose), lysozyme and acromopeptidase to break the cell walls. Then the cells were subjected to repeated freeze and thaw at -80^{o}C and 65^{o}C.

The extracted DNA was purified with phenol, chloroform and IAA and then precipitated using isopropanol.

3.5 Analytical methods

COD, BOD, TOC, NH_4^+-N, NO_2^--N, NO_3^--N and Total Nitrogen (TN) in the feed, mixed liquor supernatant and effluent were determined regularly. Sludge was also sampled to measure the MLSS and MLVSS concentrations.

Suspended Solid (SS). Total suspended solid and volatile suspended solids were measured in accordance with Standard Methods (APHA 20th Edition, 1998). Sample was dried in an oven (MEMMERT ULM 6, Schmidt Scientific, Germany) at 103 - 105°C for at least 1 hour and then ignited in a furnace (Thermolyne 48000, Omega Medical Scientific, USA) at 550°C for 20 minutes.

Biochemical Oxygen Demand (BOD). The BOD measurements were done in accordance with Standard Methods (APHA, 1998). The dissolved oxygen (DO) concentration in the samples was monitored with a DO meter (YSI, Model-58).

Chemical Oxygen Demand (COD). The Closed Reflux Method in accordance with Standard Methods (APHA 20th Edition, 1998) was used to analyze the total COD and soluble COD.

Total Organic Carbon (TOC). Total Organic Carbon Analyzer (TOC-VCSH, Shimadzu) with ASJ-V (Auto Sampler and Injector) was used to determine the organic carbon concentration of the samples. All samples were diluted to less than 25mg/L before analysis. The method used was 680^{o}C catalytically-aided combustion oxidation.

Total Nitrogen (TN). Total Nitrogen Measuring Unit (TNM-10, Shimadzu) with ASJ-V (Auto Sampler and Injector) was used to determine the total nitrogen concentration of the samples. The method used was thermal decomposition.

Ammonia Nitrogen (NH_4^+-N). NH_4^+-N was measured by using the 4500-H Automated Phenate Method with the Mark III multi-channel color meter continuous flow analysis setup (Auto Analyser Accessories, Chemlab Instrument, UK) in accordance with Standard Methods (APHA 20th Edition, 1998).

Inorganic Ions. Anions (NO_2^- and NO_3^-) were analysed using ion chromatography with Chemical Suppression Effluent Conductivity module employing ultraviolet spectrophotometric screening method recorded using a SpectraPhysics SP4290 Integrator recorded (Ion Chromatograph: Dionex DX-500, Dionex Corporation, USA) in accordance with Standard Methods (APHA 20th Edition, 1998).

Proteins. The method described by Lowry *et al.* (1951) was followed except for some slight modifications in the preparation of reagents. The first step was a biuret reaction where peptide bonds in protein react with copper in alkaline solution. The next step was a reduction of the active phosphomolybdic and phosphotungstic acids in the

reagent by the copper treated protein. The colour developed was measured spectrophotometrically at an absorbance of 650nm using HACH DR/4000 spectrophotometer to determine the concentration of proteins in the biomass.

Carbohydrates. The procedure described by Dubois *et al.* (1956) was followed using the phenol reagent as a 5% solution in water. The sample was heated with strong sulphuric acid together with the reagent to develop an orange colour. The sample was then measured spectrophotometrically at an absorbance of 490nm using HACH DR/4000 spectrophotometer.

Transmembrane Pressure and flux. The transmembrane pressure in –kPa was registered once daily from the pressure gauge which was connected to the permeate pipe from the membrane modules. The permeate flux was consistently monitored to maintain it at desired flux.

Total Phosphate (PO_4^{3-}-P). PO_4^{3-}-P was measured by HACH DR/4000 spectrophotometer coupled with HACH Kit for high range of total phosphorus (0 to 100mg/L PO_4^{3-}). The testing procedure used was the HACH method "3040".

Sludge Volume Index (SVI). Unstirred Sludge Volume Index for mixed liquor was determined in accordance with Standard Methods (APHA, 1998). 100mL measuring cylinder was used instead of 1L.

Turbidity. Turbidity for anaerobic effluent, sewage and CAS effluents was determined using Hach 2100N Turbidimeter.

Alkalinity. Alkalinity was measured by titration in accordance with Standard Methods (APHA, 1998), using 0.1N of hydrochloric acid with the use of automated-titrator (Metrohm Titrando 808)

Molecular Weight (MW) Distribution. MW distribution were determined using a 50 ml stirred ultrafiltration cell (amicon® model 8050, Millipore Corporation, USA) using 44.5mm Millipore disc ultrafiltration membranes. Three membranes with nominal MWs of 100,000 (100K), 10,000 (10K) and 1,000 (1K) daltons were used in succession with the highest MW first and lowest MW last. Pure nitrogen was used to pressurize the cell. The pressure in the ultrafilter was kept constant at 30 psi. Samples taken after each of the filters were analysed to determine specific TOC.

Microscopy. Sludge samples from the reactors were viewed using Leica, MZ6, mounted with JVC TK-C1380, colour video camera. The images were subsequently analyzed using the Leica Qwin program.

3.6 Microbial characterization techniques

3.6.1 Polymerase chain reactions (PCR) amplification

Polymerase chain reaction (PCR) amplifications of the 16S rRNA genes from the extracted DNA were performed using ExTaq^{TM} PCR kit (TaKaRa Bio Inc.). The detailed sequence of each set of primers is summarized in Table 3.6.

Table 3.6 Primers used for PCR

Primers	Domain	Sequences (5' → 3')	Remarks
47f-Cy5	*Bacteria*	Cy5-CYT AAC ACA TGC AAG TCG	Forward
927r	*Bacteria*	ACC GCT TGT GCG GGC CC	Reverse
Arch_344f	*Archaea*	Cy5-ACG GGG YGC ASC AGG CGC GA	Forward
Arch_1115r	*Archaea*	TGG GTC TCG CTC GTT G	Reverse

Reactions of (50 µL final) volume were initially denatured for 2 min at 94^{o}C followed by 30 cycles of 94^{o}C for 1 min, 55^{o}C for 1 min and 72^{o}C for 2 min. This was followed by a final extension step of 72^{o}C for 10 min.

3.6.2 PCR product purification

The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH) making use of micro centrifuges. This was carried out to concentrate the PCR products and to remove any unwanted substances, so that T-RFLP can be carried out effectively.

3.6.3 Terminal-restriction fragment length polymorphism (T-RFLP)

T-RFLP was performed by digesting the purified fluorescent-labeled PCR products with *Msp*I, *Rsa*I, and *Hha*I for 16S rRNA gene. All digestions were carried out by incubating at 37^{o}C for overnight. The digested products were loaded into a CEQ 8000 automated sequencer (Beckman Coulter) and the T-RFs length were determined by comparison with internal DNA standards (60 – 640 bp) using the CEQ 8000-genetic analysis system software.

CHAPTER FOUR RESULTS AND DISCUSSION

The post-treatment study for anaerobic effluents was carried out in 2 phases with different focuses.

In phase 1, the focus was on organic removal and nitrification performance of post-treatment for anaerobic effluents. Performances of CAS and MBR were compared at HRTs of 8 and 4 h; with anaerobic pre-treatments operated at HRTs of 16 and 6 h, respectively. Various parameters such as COD, BOD_D, NH_4^+-N, NO_2^--N, and NO_3^--N were analyzed frequently to monitor the performance of post-treatments over time.

In phase 2, the focus was shifted to nitrogen removal through biological nitrification-denitrification for anaerobic effluents. As carbon source is an important factor that affects the rate of denitrification, different percentages of raw sewage were added to vary carbon concentration in anaerobic effluents. The chosen percentages of raw sewage addition were 0%, 25% and 50%. Rates of denitrification were compared at different COD/N ratios.

In addition, distribution of molecular size fractions were determined to understand the degradation of dissolved organic matters in the water. Finally, 16S rRNA gene was used to study the dynamics of the microbial communities in the different post-treatment systems.

4.1 Phase 1 – Organic removal and nitrification performance

Despite good organic removal, treated effluent from anaerobic treatment can rarely meet the discharged standards. In addition, nitrification is needed as there is concern of ammonium in anaerobic effluent. Hence, post-treatment is essential. In phase 1, both CAS and MBR consisted of aerobic tank for organic oxidation and nitrification were set up for the study.

4.1.1 Biomass characteristics

SRT represents the average period of time during which the sludge has remained in the system. In a conventional secondary clarifier, only the fraction of activated sludge that settles as floc can be retained. Whereas, for a MBR, all biomass that is larger than the membrane cut off size is retained. The loss of biomass in MBR is minimal; hence a high SRT is achievable.

To achieve high degree of nitrification, a higher SRT is preferable so as to promote the growth of slow-growing nitrifier. Study by Ng and Hermanowicz (2005) shows that complete nitrification was achieved at a SRT of 5 d, only partially at a SRT of 2.5 d and ceased when the SRT was less than 2.5 d.

The SRTs for the aeration tanks of CASs and MBRs for the study were maintained at 10 and 20 d, respectively. Due to the different SRT employed in CASs and MBRs, the operating conditions for both systems were slightly different and a direct comparison might not be possible. However, with the aim of accessing the possibility of replacing

CAS with MBR, it is more appropriate to simulate the real-world condition where higher SRT is possible for MBR.

To monitor the growth of biomass, MLVSS concentrations were measured regularly. Floc sizes for CAS and MBR were observed periodically while SVI was measured for CAS to quantify the settling characteristics of activated sludge. In addition, the effect of EPS concentration on sludge flocculation was discussed.

4.1.1.1 Biomass concentrations

Figures 4.1, 4.2 and 4.3 show the MLVSS profiles for the CASs and MBRs operating at HRTs of 8 and 4 h for the three different anaerobic effluents. The concentrations of MLVSS in MBR were observed to be higher than CAS throughout the study. Significant difference in MLVSS between MBR and CAS was found when both systems were operating at a lower HRT of 4 h.

At the lower HRT of 4 h, MLSS of MBR with higher SRT of 20 d was observed to be approximately 50% more than the MLSS of CAS. High SRT is one of the main advantages of MBR, considering that in CAS, long SRT is impossible due to bad settling ability of sludge at high concentration and washout of suspended solids with the effluent. Typical values for MLSS concentration in MBR vary from 10,000 to 25,000 mg MLSS / l, while in CAS the concentration is around 1,500 to 5,000 mg MLSS / l (Rosenberger *et. al.,* 2001, Tchobanoglous and Burton, 2004).

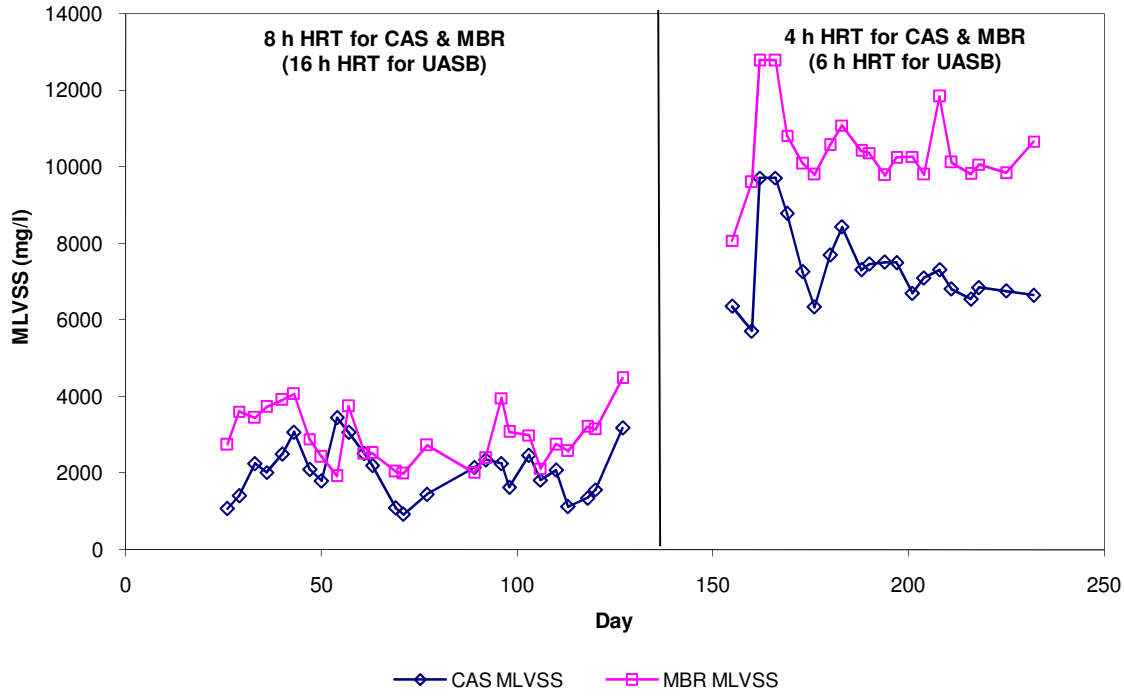


Figure 4.1 MLVSS profiles for CAS and MBR operating at different HRTs treating UASB effluents.

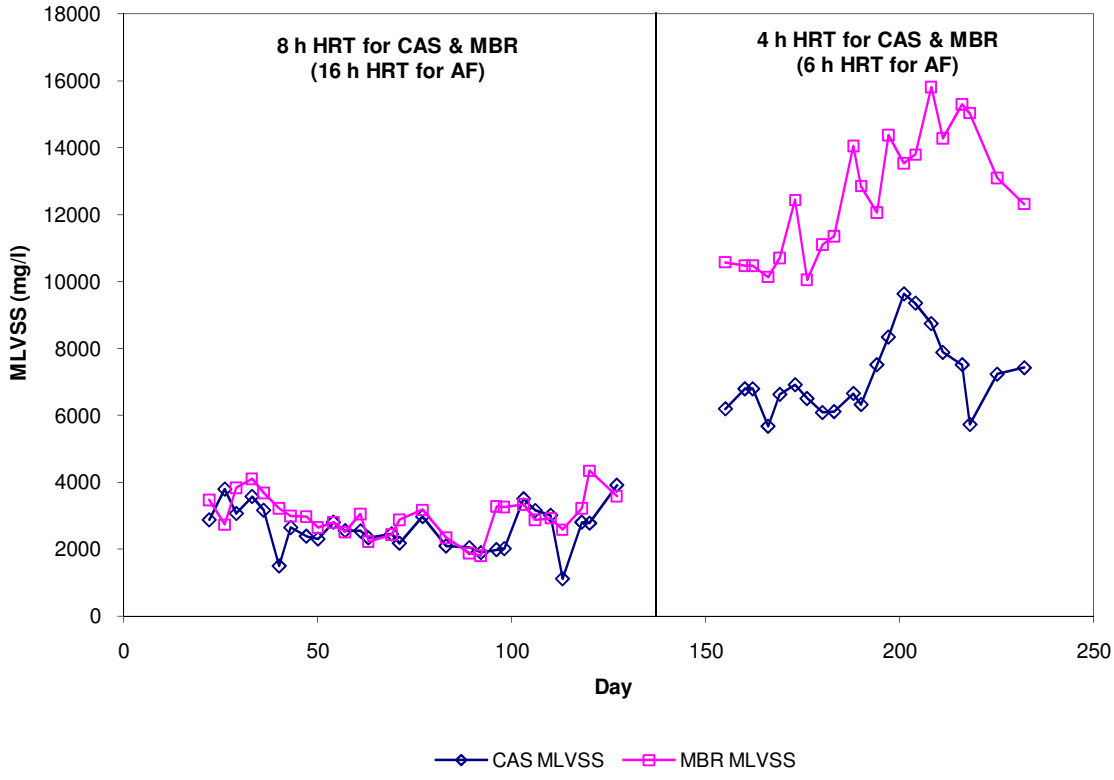


Figure 4.2 MLVSS profiles for CAS and MBR operating at different HRTs treating AF effluents.

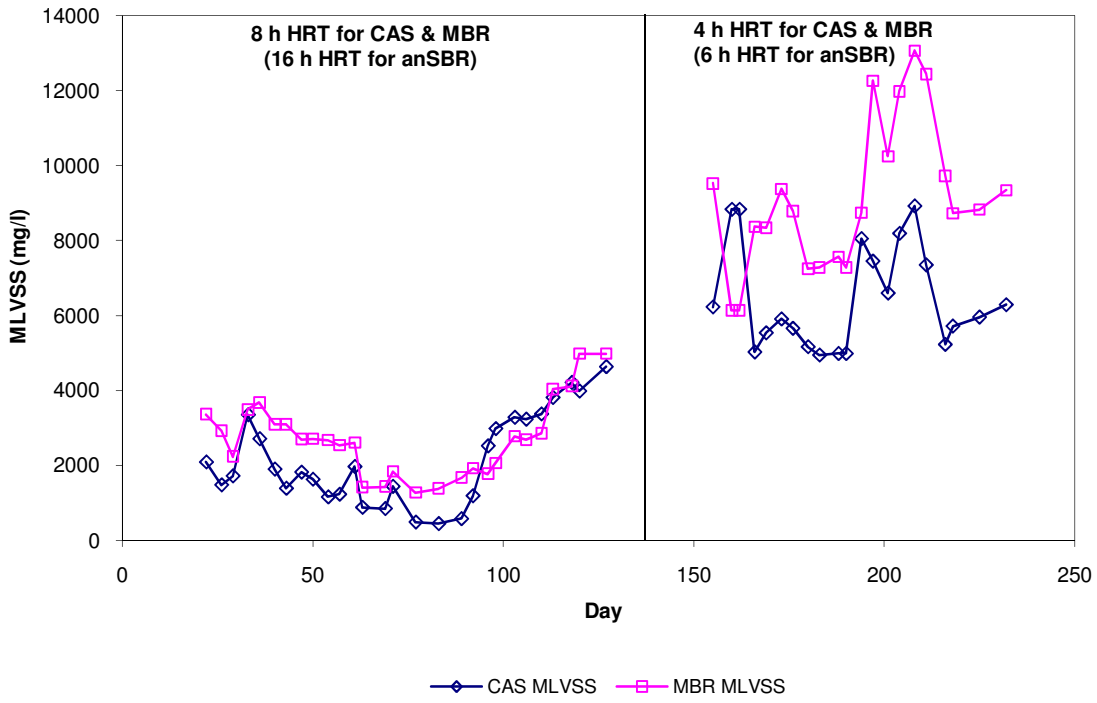


Figure 4.3 MLVSS profiles for CAS and MBR operating at different HRTs treating anSBR effluents.

Tables 4.1 and 4.2 show the average MLSS and MLVSS concentrations of CASs and MBRs treating effluents from the three anaerobic processes.

Table 4.1 MLSS* concentrations of CAS and MBR

(in mg/l)	Anaerobic pre-treatment process		
	UASB	AF	AnSBR
CAS			
8-h HRT	2920 ± 930	3950 ± 1050	4200 ± 1300
4-h HRT	10025 ± 500	10600 ± 1450	9900 ± 1400
MBR			
8-h HRT	4160 ± 1050	4440 ± 1050	4400 ± 1220
4-h HRT	14600 ± 650	19300 ± 1600	14400 ± 1800

* HRT of anaerobic pre-treatment was 16 h when aerobic post-treatment was 8 h, while 6 h when aerobic post-treatment was 4 h

Table 4.2 MLVSS* concentrations of CAS and MBR

(in mg/l)	Anaerobic pre-treatment process		
	UASB	AF	AnSBR
CAS			
8-h HRT	1875 ± 600	2500 ± 700	2825 ± 750
4-h HRT	7050 ± 360	7700 ± 1200	6900 ± 1100
MBR			
8-h HRT	2735 ± 700	2900 ± 650	3000 ± 750
4-h HRT	10250 ± 570	13880 ± 1200	9930 ± 1430

* HRT of anaerobic pre-treatment was 16 h when aerobic post-treatment was 8 h, while 6 h when aerobic post-treatment was 4 h

The average biomass concentrations in CAS systems were observed to be closed to 3000 mg/l when post-treatments were operated at HRT of 8 h. It was similar to the inoculation concentration collected from the activated sludge tank of the local wastewater treatment plant. Higher biomass concentrations were observed in MBR due to the higher SRT used, as represented by equation 4.1 (Tchobanoglous and Burton, 2004).

$$X = \frac{\theta_c}{\theta}\left[\frac{Y(S_0 - S)}{1 + k_d\theta_c}\right] \qquad (4.1)$$

The biomass concentrations increased tremendously when HRT for both pre- and post-treatment changed from 16 and 8 h to 6 and 4 h respectively. The increased in biomass concentrations was mainly due to higher substrate available resulted from poorer performance of the anaerobic pre-treatments. Higher SS present in anaerobic effluents and biomass washed out from anaerobic pre-treatments also loaded both CAS and MBR with higher solids concentrations.

4.1.1.2 Biomass settleability

Biomass settleability is a main concern in CAS as the effluent quality depends largely on the performance of biomass settling in the clarifier. However, it is not of concern to MBR which uses membrane to achieve solid-liquid separation. One of the important factors for biomass settleability is floc size, which EPS concentration will influence the sludge flocculation.

4.1.1.2.1 Floc sizes

In order to remain in the CAS system, the biomass has to be able to settle well in the clarifier. The dispersed biomass that cannot settle will be washed out with the effluent. Hence, biomass flocculation is important to increase the mass of flocs for CAS. In plate 4.1(a), the CAS flocs were observed to be larger and stronger. In contrast, MBR flocs were smaller and weaker as shown in plate 4.1(b). The smaller and weaker flocs in MBR would be due to the high aeration used (5 l/min) which broke up the flocs. In addition, due to the complete retention of biomass within MBR, all biomass regardless of settleability were retained.

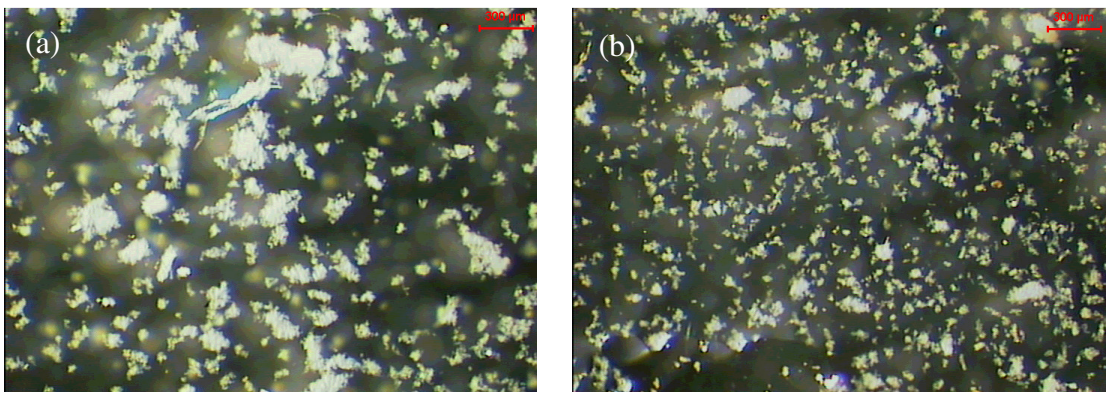


Plate 4.1 Flocs found in aeration tanks of (a) CAS and (b) MBR (bar = 300 μm).

4.1.1.2.2 EPS concentrations

Microbial cells can produce EPS which lead to floc formation by agglomeration of bacteria. The EPS is responsible for increased bridging flocculation that helps in biomass settling, which is especially crucial to the CAS process.

Specific EPS concentrations (DOC normalized with biomass concentration) were measured to quantify biomass EPS production. Protein, carbohydrates and humic substances are the dominant components typically found in EPS (Jahn and Nielsen, 1998; Liao *et. al.* 2001). In this study, the specific concentrations of protein and carbohydrates in EPS were measured. Table 4.3 provides the summary of biomass EPS contents for both CASs and MBRs at HRT of 8 and 4 h.

Table 4.3 Summary of biomass EPS contents for both CAS and MBR

Pre-treatment	**UASB**		**AF**		**AnSBR**	
Post-treatment	CAS	MBR	CAS	MBR	CAS	MBR
8 h HRT						
F/M (mg COD/ mg VSS)	0.071	0.072	0.114	0.122	0.104	0.100
SVI	14.1±6.13	-	16.5±4.37	-	14.5±5.45	-
Specific EPS (mg DOC/ g VSS)	3.85±2.07	4.22±1.27	4.97±0.93	4.17±0.61	5.99±1.39	4.13±1.14
Protein (mg BSA/ g VSS)	9.95±3.30	13.10±3.41	14.35±2.90	13.62±2.64	18.06±5.21	12.12±3.62
Carbohydrates (mg glucose/ g VSS)	3.14±1.74	3.90±0.89	4.25±0.83	4.43±0.79	5.47±1.62	4.13±1.14
4 h HRT						
F/M (mg COD/ mg VSS)	0.048	0.037	0.057	0.035	0.039	0.029
SVI	62.6±18.0	-	55.4±14.9	-	42.5±10.6	-
Specific EPS (mg DOC/ g VSS)	5.86±2.09	4.86±0.67	9.04±2.85	5.84±1.56	7.27±2.07	5.16±2.99
Protein (mg BSA/ g VSS)	17.65±1.09	9.51±2.03	21.18±6.55	14.33±3.53	13.10±4.48	12.92±4.87
Carbohydrates (mg glucose/ g VSS)	6.30±1.61	4.21±1.31	7.24±3.66	5.26±1.56	4.49±1.38	5.16±2.99

Liao *et al.* (2001) found that at higher SRTs, with a lower F/M ratio, the level of carbohydrate in floc EPS declined as an indication of the available carbon in wastewater. The values of carbohydrates per VSS obtained from the 8 h HRT coincided with Liao's finding, where carbohydrates were found to decrease with lower F/M.

Soluble microbial products (SMP), which are composed of a variety of organic compound released from microorganisms as a result of their metabolic activity (Barker and Stuckey, 1999), are considered to be major foulants of membranes used in MBRs. The SMP was characterized by measurement of carbohydrates and proteins. Hence, increases in carbohydrates and proteins' concentration would contribute the evolution of fouling in MBR.

The decrease of F/M ratios when the HRT was reduced to 4 h showed the increase of specific EPS in the range of 15 to 40% in MBR and 21 to 80% in CAS, as presented in Table 4.3. This coincided with the findings reported by Pavoni *et. al*. (1972) and Sheintuch *et. al.* (1986), where larger amount of EPS was detected when endogenous metabolism predominates at low F/M ratios compared to conditions when high F/M ratios were supplied.

However, direct comparison with the other findings was difficult as no trend in the EPS production was observed in the three different post-treatments. In addition, the other findings were quite different owing to the use of different biomass and feed wastewater in different studies.

The settleability of the biomass at lower HRT deteriorated as indicated by the increase of SVI by twice. The concentrations of biomass increased tremendously at lower HRT while higher specific EPS were produced. The deterioration could be attributed to bulking where aggregates were not compacted and formed loose, low density floc (Lau *et. al.*, 1984).

4.1.2 Organic removal performance

Organic removal performances for both the systems were monitored by COD, BOD and SS concentrations in feed and treated effluent regularly.

4.1.2.1 Chemical oxygen demand (COD) and biochemical oxygen demand (BOD)

Figures 4.4, 4.5 and 4.6 show the tCOD profiles of feed, CAS and MBR effluents for the three different pre-treatments. In the MBR effluent, tCOD was observed to be equal to sCOD and tBOD equal to sBOD since the membranes were able to filter out all solids effectively and no suspended solids were detected in the effluent. Table 4.4 summarizes the COD, BOD and TOC of feed, CAS and MBR effluents for different post-treatments.

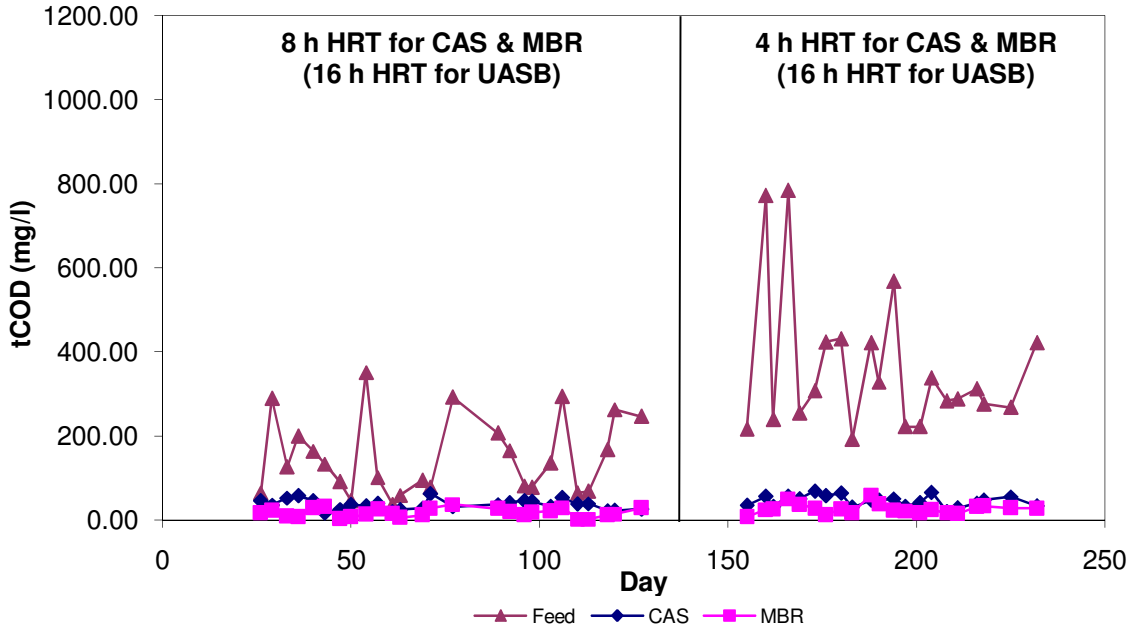


Figure 4.4 tCOD profiles for feed, CAS and MBR effluents operating at different HRT treating UASB effluents.

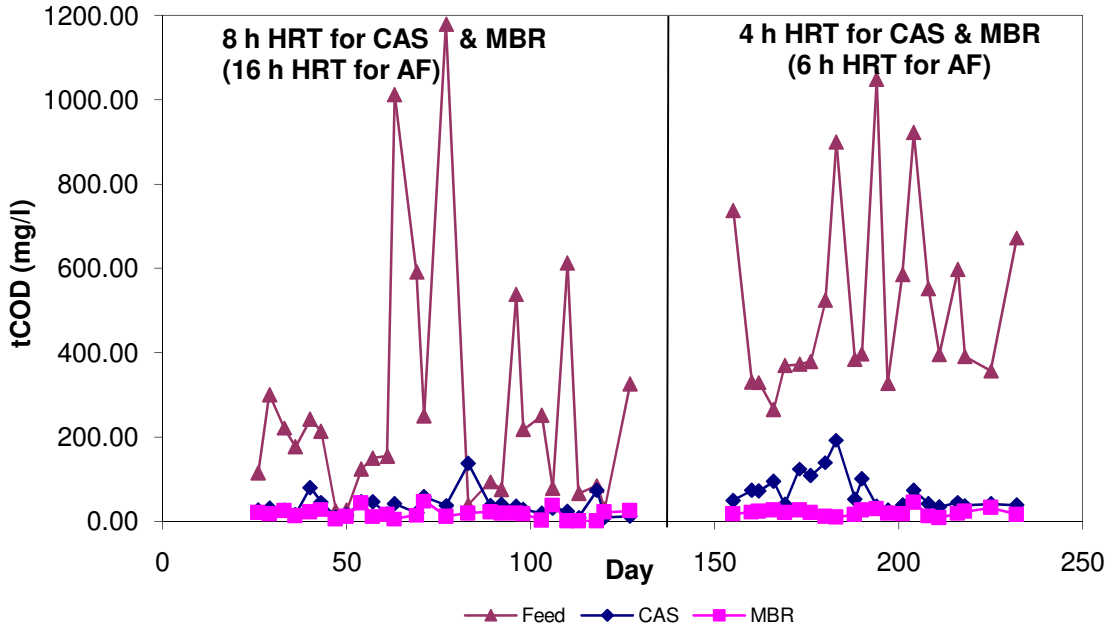


Figure 4.5 tCOD profiles for feed, CAS and MBR effluents operating at different HRT treating AF effluents.

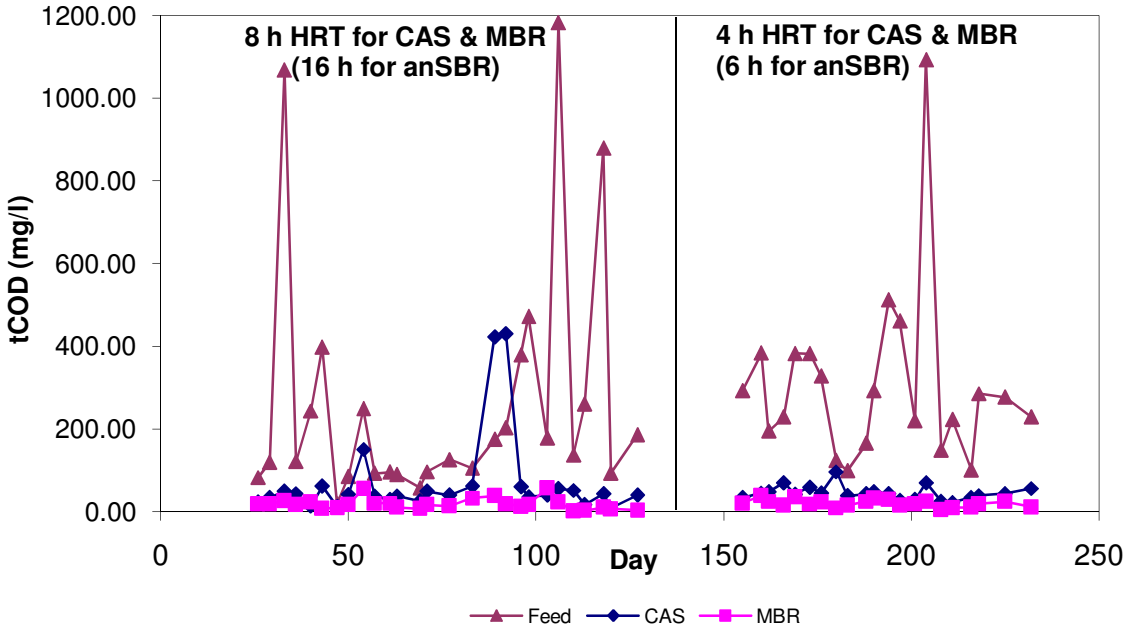


Figure 4.6 tCOD profiles for feed, CAS and MBR effluents operating at different HRT treating anSBR effluents.

Table 4.4 COD, BOD and TOC of feed, CAS and MBR effluents for different post-treatments

Pre-treatment	**UASB**			**AF**			**anSBR**		
	feed	**CAS**	**MBR**	**Feed**	**CAS**	**MBR**	**feed**	**CAS**	**MBR**
8 h HRT									
tCOD	141.3 ± 88.1	36.5 ± 13.6	17.7 ± 10.2	288.4 ± 194.6	32.7 ± 18.2	17.0 ± 13.2	236.3 ± 168.1	40.0 ± 14.1	17.4 ± 14.4
sCOD	46.4 ± 15.9	18.5 ± 9.8		46.0 ± 29.9	17.3 ± 9.8		47.3 ± 17.1	24.0 ± 13.6	
tBOD	23.0 ± 2.21	5.69 ± 0.75	3.07 ± 1.19	40.0 ± 15.5	3.90 ± 0.54	1.27 ± 0.20	61.9 ± 3.94	4.29 ± 1.81	1.22 ± 0.41
sBOD	13.2 ± 8.52	2.04 ± 0.44		6.71 ± 0.84	1.54 ± 0.10		8.98 ± 2.13	1.93 ± 0.40	
TOC	15.9 ± 5.54	10.3 ± 4.48	7.02 ± 1.22	20.7 ± 10.1	8.99 ± 2.55	6.87 ± 1.29	19.3 ± 5.73	10.4 ± 2.59	7.21 ± 1.54
DOC	10.3 ± 1.80	7.36 ± 1.31		10.5 ± 1.77	7.07 ± 1.35		10.6 ± 1.90	7.78 ± 1.25	
COD/BOD	6.14			7.21			3.82		
4 h HRT									
tCOD	328.7 ± 99.2	42.4 ± 12.9	27.5 ± 12.4	527.5 ± 185.2	42.5 ± 9.5	22.6 ± 9.9	300.7 ± 171.9	40.3 ± 13.9	18.9 ± 8.7
sCOD	60.4 ± 13.0	28.3 ± 18.8		68.8 ± 14.4	25.0 ± 10.4		49.4 ± 11.4	19.9 ± 8.4	
tBOD	69.6 ± 13.4	5.49 ± 3.41	1.28 ± 0.56	171.1 ± 56.09	5.94 ± 1.71	1.20 ± 0.98	57.9 ± 28.2	6.99 ± 3.08	0.91 ± 0.38
sBOD	10.9 ± 3.10	0.98 ± 0.27		18.6 ± 4.93	1.01 ± 0.28		10.3 ± 4.52	0.84 ± 0.14	
TOC	30.0 ± 7.42	10.2 ± 3.60	8.83 ± 1.86	33.4 ± 7.88	10.3 ± 4.27	8.06 ± 1.88	24.5 ± 7.01	11.7 ± 9.32	7.62 ± 2.35
DOC	14.4 ± 3.07	8.32 ± 1.73		18.2 ± 3.96	7.97 ± 1.83		14.1 ± 3.05	7.79 ± 1.95	
COD/BOD	4.72			3.09			5.19		

Note: All in units of mg/l except COD/BOD ratio, which is unitless

Despite the variation in influent wastewater quality, all CAS and MBR systems were able to produce effluent of consistently good quality (less than 50 mg/L in tCOD and less than 10 mg/L in tBOD). All effluents from CAS and MBR were able to meet the discharge requirement to controlled watercourse (tCOD < 60mg/l and tBOD < 20mg/l) of Singapore (NEA, 2005). The removal performances showed that both systems had very little sensitivity to the fluctuations in influent wastewater quality. It was especially true for MBR; however CAS might still experience poor removal performance when the sludge settleability was affected.

MBR outperformed CAS for both COD and BOD removal since performance of MBR is not dependent on the settleability of biomass. In addition, the higher SRT coupled with higher MLVSS concentration for MBR also allowed less biodegradable organic compounds to be broken down more readily. The performance of MBR was consistent for various HRTs as reported by Côté *et al*. (1997) that the performance of MBR appeared to be relatively insensitive to HRTs within values between 2 to 24 h and corresponding to a very high removal percentage.

4.1.2.2 Suspended solids (SS)

MBR were able to achieve complete solid-liquid separation, therefore no SS was observed in the effluent of MBR. Figures 4.7, 4.8 and 4.9 show the SS profiles for feed and CAS effluents with different pre-treatment systems. In addition, Table 4.5 shows the TSS and VSS concentrations for both feed and CAS effluent.

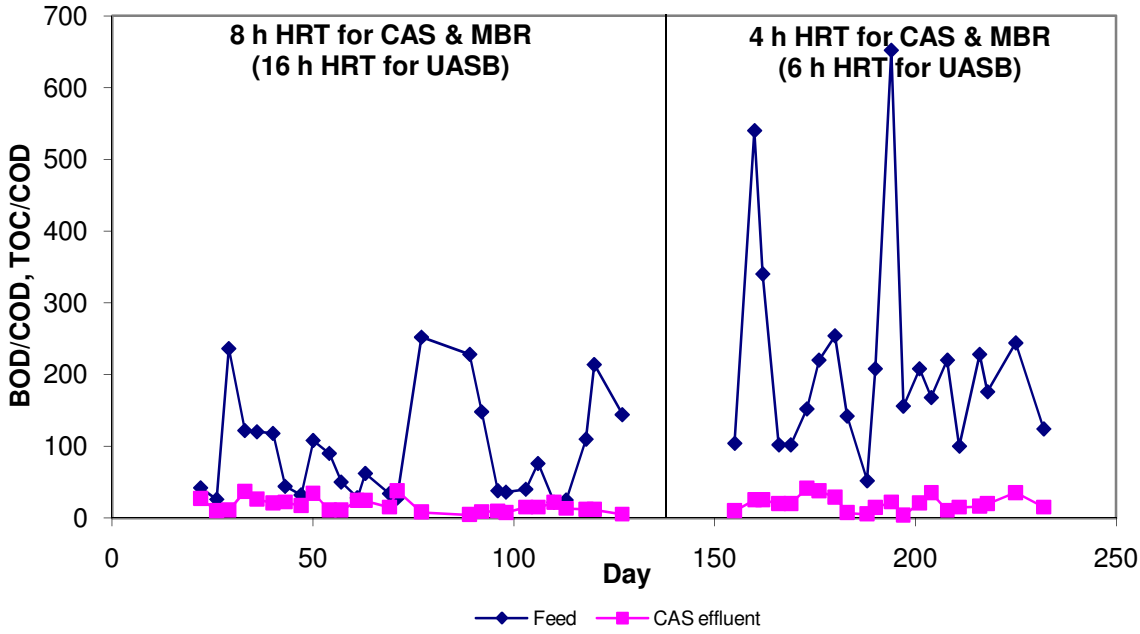


Figure 4.7 SS profiles for feed and CAS effluent operating at different HRTs treating UASB effluents.

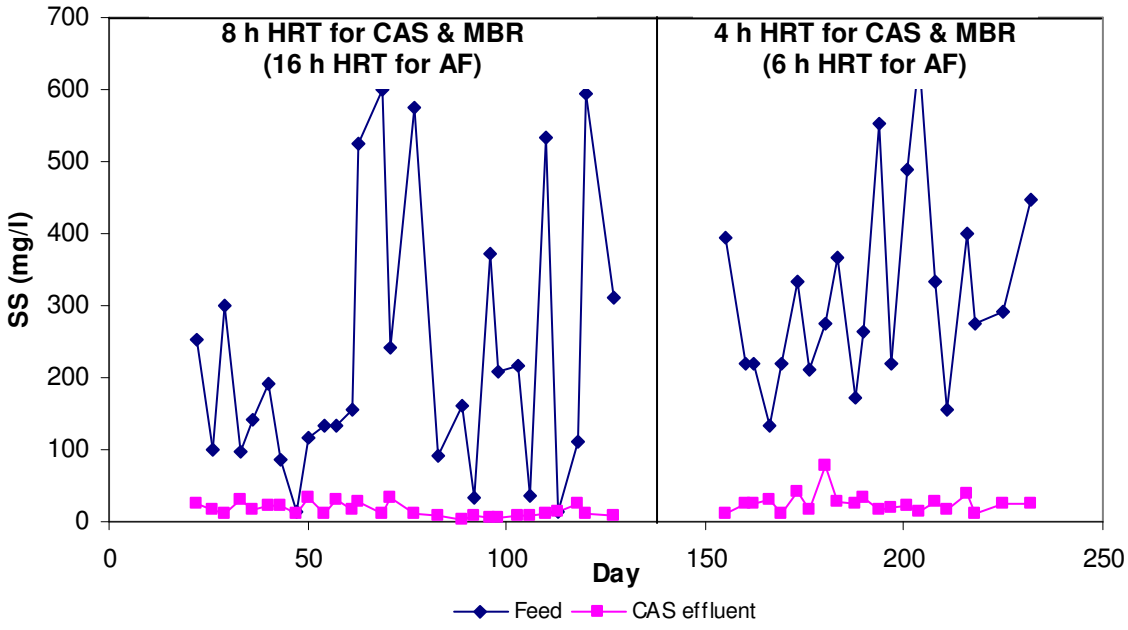


Figure 4.8 SS profiles for feed and CAS effluent operating at different HRTs treating AF effluents.

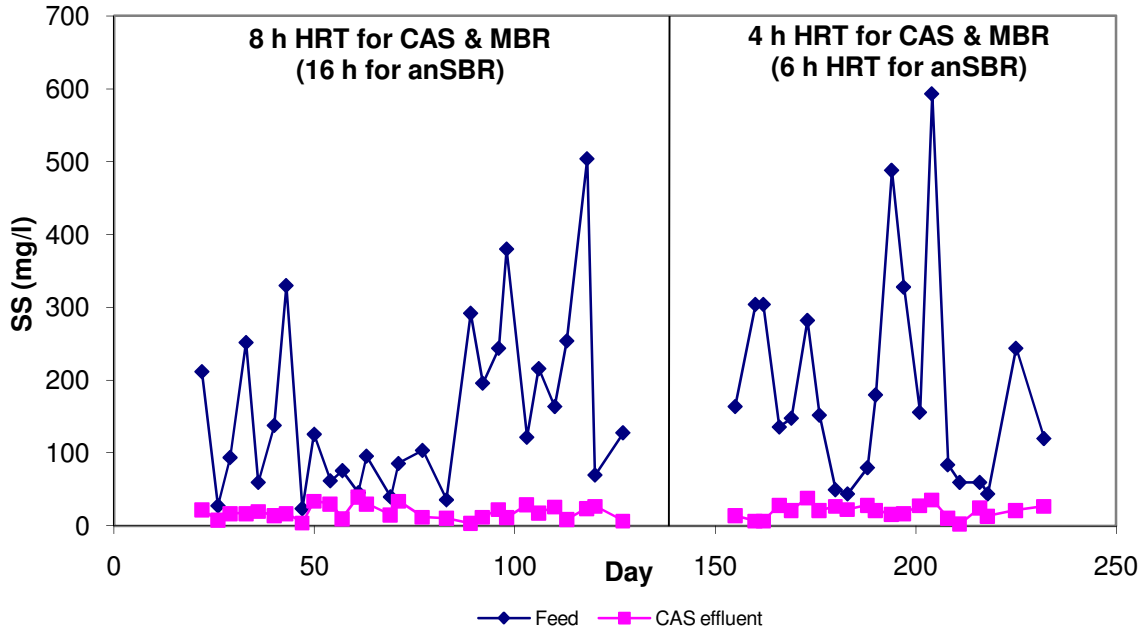


Figure 4.9 SS profiles for feed and CAS effluent operating at different HRTs treating anSBR effluents.

The post-treatments were able to achieve effluent SS concentration of less than 30 mg/L to meet Singapore standard for discharge to controlled watercourse (NEA, 2005) despite the variation in SS in feed except for anSBR during the 8 h HRT operation. It was due to the change of HRT for anSBR from 16 to 6 h on Day 89. Serious biomass wash-out happened in anSBR that resulted in high biomass transferred to the anSBR post-treatment. Biomass was unable to flocculate well; pin flocs were found in the clarifier and affected the SS concentration of the effluent.

Biomass wash-out from the anaerobic pre-treatments occurred occasionally. When this happened, the SS concentrations in influent of post-treatments would be double as compared to average concentration. CAS could not handle it and would result in high SS in effluent. Hence, control must be in place for anaerobic pre-treatment to minimize the biomass wash-out that can cause sudden high SS loadings to CAS. Nonetheless, biomass wash-out will not affect the SS removal performance of MBR.

Table 4.5 TSS and VSS of feed and CAS effluent

(in mg/l)	UASB		AF		anSBR	
	feed	CAS	feed	CAS	feed	CAS
8 h HRT						
TSS	88.4 ± 80.4	15.6 ± 15.0	179.7 ± 96.0	14.1 ± 12.0	115.3 ± 59.0	27.2 ± 15.6
VSS	66.4 ± 60.0	10.1 ± 9.2	129.7 ± 69.2	9.4 ± 7.0	91.4 ± 49.0	11.5 ± 4.5
4 h HRT						
TSS	211.3 ± 150.0	18.5 ± 11.7	370.3 ± 185.1	15.1 ± 7.8	203.1 ± 180.0	20.6 ± 9.2
VSS	154.0 ± 124.0	13.2 ± 8.8	285.0 ± 144.6	19.4 ± 11.4	144.7 ± 132.0	13.8 ± 6.8

4.1.2.3 COD/BOD ratio

COD/BOD ratios provide information about the biodegradability of wastewater. Tchobanoglous and Schroeder (1985) reported that the COD/BOD_5 ratios varied from 1.25 to 2.5 for untreated domestic wastewater. If COD/BOD5 ratio is above 3, the wastewater can be degraded only slowly or with difficulty.

In the anaerobic-aerobic process, domestic wastewater would first go through the anaerobic process where large portion of biodegradable COD are consumed, hence leaving an anaerobic effluent that is less biodegradable. The COD concentration for the aerobic post-treatment would be lower; however the fractions of the less biodegradable COD would increase.

The COD/BOD ratios for the anaerobic effluents are shown in Table 4.4. All the ratios were found to be above 3; therefore biodegradation might be slowly or with difficulty. Despite this, both CAS and MBR were still able to achieve good quality effluents.

4.1.3 Nitrification performance

Nitrification is the two steps biological process in which ammonia (NH_4^+-N) is oxidized to nitrite (NO_2^--N) and nitrite is oxidized to nitrate (NO_3^--N) by two types of distinctly different autotrophic bacteria, Nitrosomonas and Nitrobacter. In this study, nitrification is achieved along with organic removal in the same single-sludge process.

The conditions for maximum growth of nitrifying bacteria in the reactor should be provided, because their growth rate is lower than that of the heterotrophic bacteria which will compete with them for organics.

Several factors that will affect the nitrification rates are pH, DO concentration and SRT. To ensure optimal nitrification rates, pH of aeration tanks were controlled between 6.8 and 7.5, while DO concentrations were kept above 2 mg/l. High SRT of 10 and 20 d were controlled for CAS and MBR, respectively.

4.1.3.1 Ammonia removal performance

Tables 4.6 and 4.7 show the average total nitrogen (TN) removal efficiency and effluent quality of CASs and MBRs operated at 8 and 4 h HRT. A near complete

nitrification performance was observed most of the time for all the CAS and MBR systems except for a few incidents that affected the nitrification.

On Day 98 during 8 h HRT operation, a drop of biomass concentration was observed in the CAS system treating UASB effluent. Biomass concentration dropped from 2080 to 1120 mg/l. Only 75% NH_4^+-N removal was achieved. The loss of nitrifiers most probably affected the nitrification. According to Cicek et al. (2001), there is a decrease in nitrification rate at very low SRT (2 days), supposedly due to a partial loss of nitrifying microorganisms. However, as the biomass concentration started to increase, near complete nitrification was achievable 7 days later.

On Day 110, anSBR suffered a pH shock and resulted in an acidic effluent. However, the buffer pump of the MBR system was not activated to dose in buffer to neutralize the acidic effluent from anSBR. Therefore the pH of the MBR dropped to 4, which hindered nitrification. Only 70% NH_4^+-N removal was achieved. For low pH, the predominated CO_2 can be stripped from the mixed liquor by the aeration which would result in alkalinity acarcity (Villaverde *et al.*, 1997). However, nitrification performance was recovered to the original level when the pH problem was rectified.

TN removal efficiency was rather low for both systems operated at 8- as well as 4- h HRT. As NH_4^+-N oxidized to NO_2^--N and to NO_3^--N, there was only a conversion of nitrogen to different forms, and most of the nitrogen would still remain in the effluent. The small amount of TN lost was most likely due to assimilation by biomass for growth and the removal of SS.

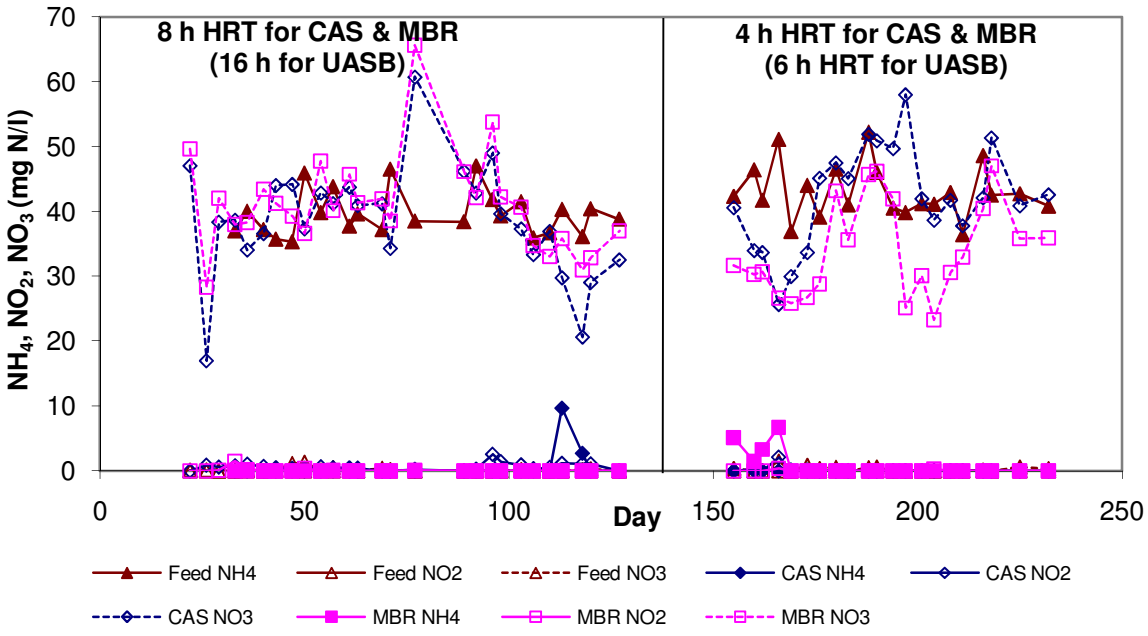


Figure 4.10 NH_4^+, NO_2^- and NO_3^- concentrations for feed, CAS and MBR effluents operating at different HRTs treating UASB effluents.

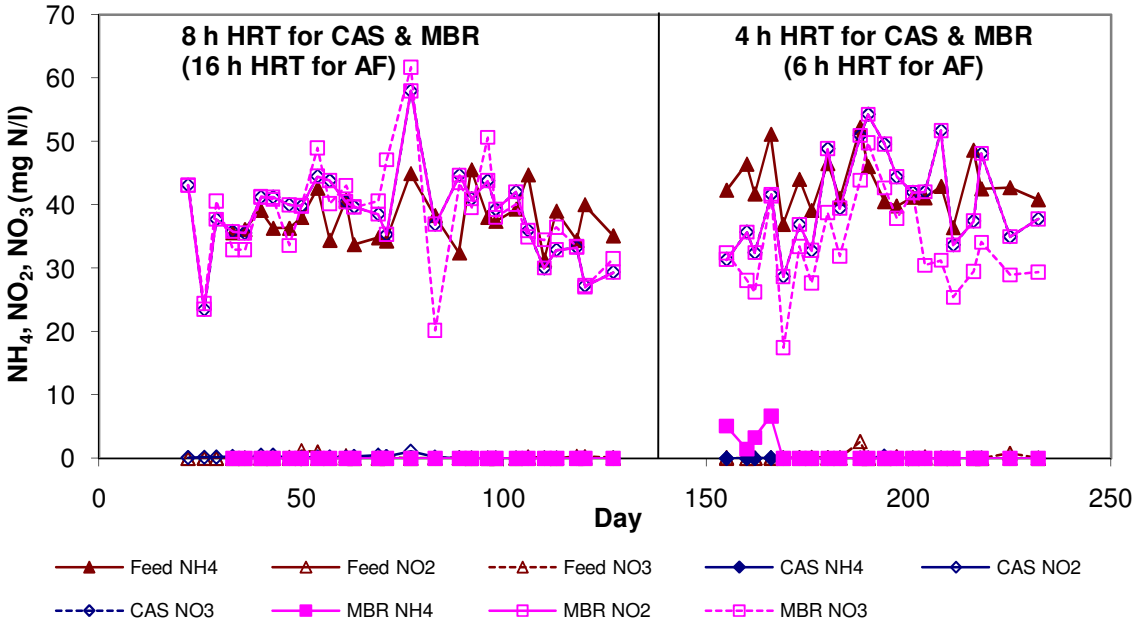


Figure 4.11 NH_4^+, NO_2^- and NO_3^- concentrations for feed, CAS and MBR effluents operating at different HRTs treating AF effluents.

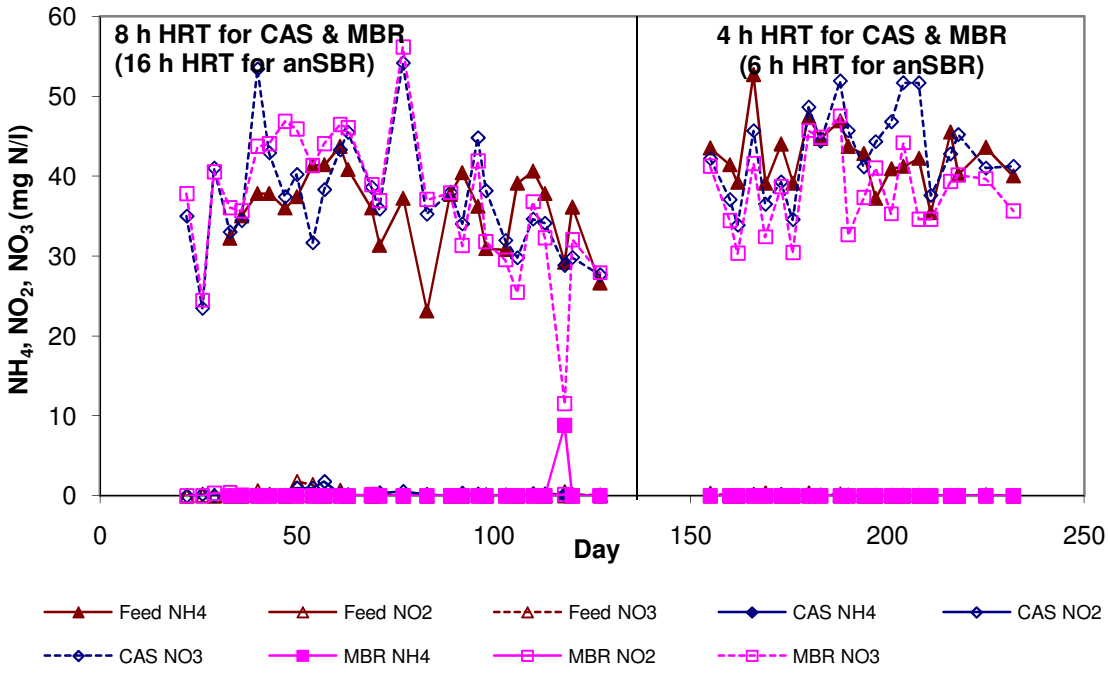


Figure 4.12 NH_4^+, NO_2^- and NO_3^- concentrations for feed, CAS and MBR effluents operating at different HRTs treating anSBR effluents.

Table 4.6 Average TN removal efficiency and effluent quality of CASs and MBRs at 8 h HRT.

(in mg/l)	Anaerobic pre-treatment process		
	UASB	AF	anSBR
Influent			
TN	36.3 ± 8.8	45.7 ± 16.82	35.4 ± 6.2
NH_4^+-N	40.0 ± 3.3	37.9 ± 4.4	35.2 ± 5.6
NO_2^--N	N.D.	N.D.	0.01 ± 0.02
NO_3^--N	0.08 ± 0.12	0.11 ± 0.14	0.11 ± 0.19
CAS			
TN	32.4 ± 5.4	32.0 ± 3.6	31.0 ± 4.1
NH_4^+-N	0.73 ± 2.40	N.D.	N.D.
NO_2^--N	0.68 ± 0.68	0.12 ± 0.27	0.15 ± 0.15
NO_3^--N	38.5 ± 9.0	38.0 ± 7.2	34.3 ± 11.3
TN removal efficiency (%)	3.89 ± 7.96	15.6 ± 21.4	10.8 ± 10.4
MBR			
TN	32.6 ± 3.6	31.1 ± 3.4	28.9 ± 5.1
NH_4^+-N	N.D.	N.D.	N.D.
NO_2^--N	0.01 ± 0.02	0.01 ± 0.04	0.01 ± 0.03
NO_3^--N	41.2 ± 8.5	38.7 ± 9.3	34.7 ± 9.9
TN removal efficiency (%)	4.0 ± 9.3	19.9 ± 22.5	16.9 ± 14.4

N.D. – non detectable

Table 4.7 Average TN removal efficiency and effluent quality of CASs and MBRs at 4 h HRT

(in mg/l)	Anaerobic pre-treatment		
	UASB	AF	anSBR
Influent			
TN	47.4 ± 5.8	50.6 ± 5.4	45.1 ± 3.3
NH_4^+-N	42.9 ± 4.2	39.0 ± 3.4	41.6 ± 3.3
NO_2^--N	N.D.	N.D.	N.D.
NO_3^--N	0.18 ± 0.25	0.31 ± 0.77	0.03 ± 0.06
CAS			
TN	41.7 ± 2.5	37.1 ± 0.84	41.5 ± 2.4
NH_4^+-N	N.D.	N.D.	N.D.
NO_2^--N	N.D.	0.03 ± 0.10	N.D.
NO_3^--N	41.4 ± 14.4	43.6 ± 7.0	44.5 ± 4.5
TN removal efficiency (%)	11.4 ± 6.0	26.1 ± 7.3	7.9 ± 2.0
MBR			
TN	38.3 ± 3.0	29.2 ± 0.7	37.6 ± 2.0
NH_4^+-N	N.D.	0.39 ± 1.47	N.D.
NO_2^--N	0.02 ± 0.08	0.06 ± 1.06	0.01 ± 0.03
NO_3^--N	36.3 ± 8.1	35.2 ± 7.6	38.8 ± 4.4
TN removal efficiency (%)	18.7 ± 4.5	41.8 ± 7.5	16.4 ± 2.4

N.D. – non detectable

4.1.4 Membrane performance

4.1.4.1 Membrane fouling

Flux of MBRs was kept constant to achieve consistent HRT; therefore fouling was indicated by increase in transmembrane pressure (TMP). Figures 4.13, 4.14 and 4.15

show the observed TMP profiles for MBRs treating 3 different anaerobic effluents. Severe fouling was not observed throughout the study at 8 h HRT. It could be due to the low effluent flow rate and the high aeration that was provided at the bottom of membranes to scour off the biomass that attached to the membranes. Besides that, the intermediate suction also helped to prevent membrane fouling.

However, Figure 4.13 shows that noticeable membrane fouling, for MBR operated at HRT of 4 h treating effluent from UASB, was first observed on Day 97. Since then, the membrane TMP increased rapidly until Day 114 when the membranes were removed for chemical cleaning. Severe membrane fouling was not observed for both the MBR treating effluent from AF and anSBR throughout the study.

Fouling at the MBR of UASB was most likely due to the accumulation of SS around the aeration tubes at the bottom of the reactor. The blockage of the holes for aeration prevented the evenly distribution of air which was used to scour the membranes to minimize fouling. Therefore, proper design for the air distribution system for MBR is essential.

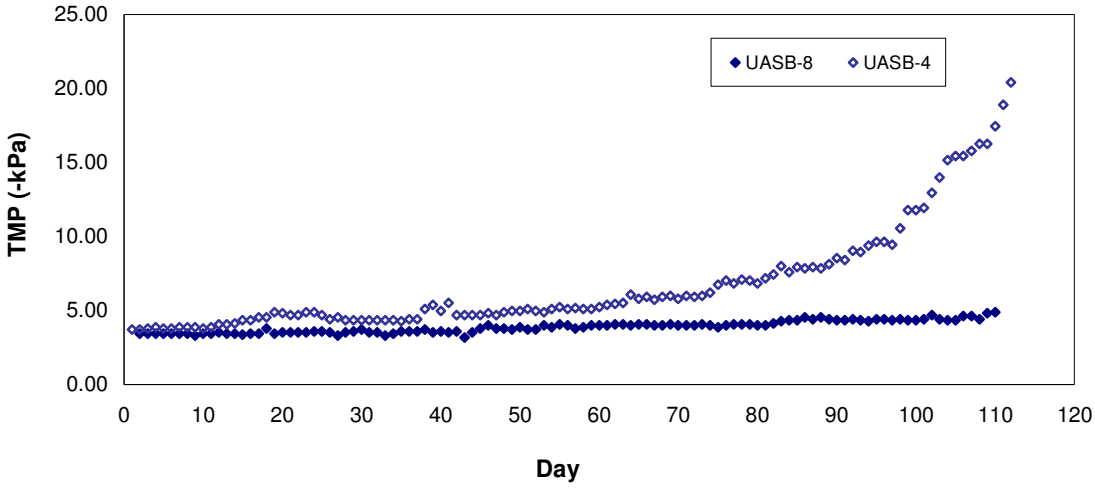


Figure 4.13 Observed TMP profiles for post-treatment operating at 8 and 4 h HRT for UASB

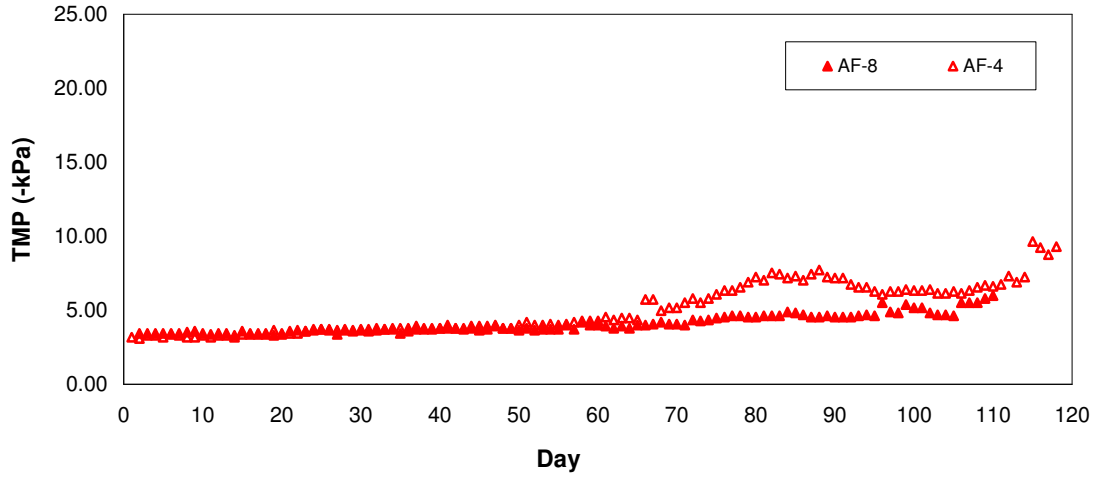


Figure 4.14 Observed TMP profiles for post-treatment operating at 8 and 4 h HRT for AF

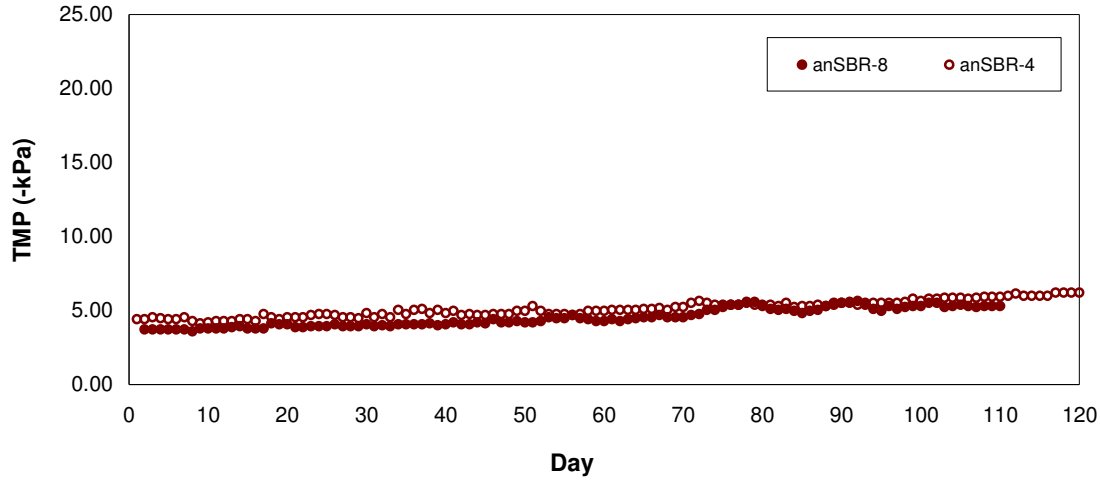


Figure 4.15 Observed TMP profiles for post-treatment operating at 8 and 4 h HRT for anSBR

4.2 Phase 2 - Nitrogen removal performance

Denitrification is the biochemical reduction of nitrate to nitric oxide, nitrous oxide and nitrogen gas. A wide range of heterotrophic and autotrophic bacteria, which can shift between oxygen respiration and nitrogen respiration, are capable of denitrification. In order to have denitrification, the nitrogen must be in one of its oxidized forms, NO_3^- or NO_2^-. Therefore, denitrification is frequently coupled with nitrification, which is needed to create the oxidized nitrogen.

Pre-anoxic denitrification process was used in this study with the anoxic tank preceded the aeration tank. Nitrate produced in the aeration tank was recycled back to the anoxic tank. Denitrification took place in the anoxic tank where organic substrate in the influent provided electron donor for oxidation-reduction reactions using nitrate.

Limitation of organic loadings in influent to aeration zone of the reactor is desirable to facilitate the growth of nitrifying bacteria as it reduces the competition from heterotrophic bacteria for oxidation. However, denitrification intensity depends on carbon available. Hence, the carbon to nitrogen ratio in the influent must be high enough to ensure efficient denitrification of nitrate arisen from the nitrification process, while low enough to encourage growth of nitrifying bacteria to achieve full nitrification.

Anaerobic treatment reduces COD; however nitrogen concentration is largely unaffected. Therefore COD/N ratio is reduced after the anaerobic treatment. To access the effect of anaerobic pre-treatment on the performance of nitrogen removal, studies

with 0%, 25% and 50% raw sewage addition based on flow rate to anaerobic effluents were conducted. The main aim of the raw sewage addition was to increase the COD concentrations of influent to the post-treatment, therefore increasing the COD/N ratio. In addition, the sewage bypass could reduce the flow to pre-treatment while maximize the usage of air provided to post-treatment, especially in MBR.

HRT of post-treatments treating effluents from UASB and AnSBR was 6 h based on the total volume of anoxic and aerobic tanks, while post-treatment for AF had a HRT of 8 h. All the three pre-treatments were operated at 6 h HRT.

4.2.1 100% anaerobic effluents

In this condition, only anaerobic effluents were fed to the post-treatment systems. No raw sewage was added. The three pre-treatment systems were operated at the similar HRT of 6 h as the previous phase of study when post-treatments were operated at 4 h HRT. Therefore the average COD concentrations of anaerobic effluents were consistent with the previous study.

The post-treatments were operated for more than 50 d and the performances for organic and nitrogen removals were monitored.

4.2.1.1 Organic removal

Figures 4.16, 4.17 and 4.18 show the tCOD concentrations of feed and effluents for the different post-treatment systems, which were fed with only anaerobic effluents. The

average values of tCOD are summarized in Table 4.9. CASs could achieve more than 85% tCOD removal while more than 90% removal was achievable by MBRs.

Côté *et al.* (1998) attributed the improved COD removal to the avoidance of biomass washout problems commonly encountered in activated sludge process, as well as to complete particulate retention by the membrane. Membrane rejection of a significant amount of soluble organic molecules and colloids makes their removal more effective due to a higher lyses activity in the reactor induced by elevated concentrations of these compounds. Higher sludge ages that are achieved by long SRTs allow more complete mineralization of biodegradable raw water organics, but also an adaptation of microorganisms to less biodegradable compounds. Therefore, biomass can acclimatize to wastewater without being restricted to fast-growing and floc-forming microorganisms.

The tCOD concentration for both CAS and MBR effluents were consistently less than 50 mg/l despite the fluctuation in the feed. The performances of the systems were comparable to the previous phase.

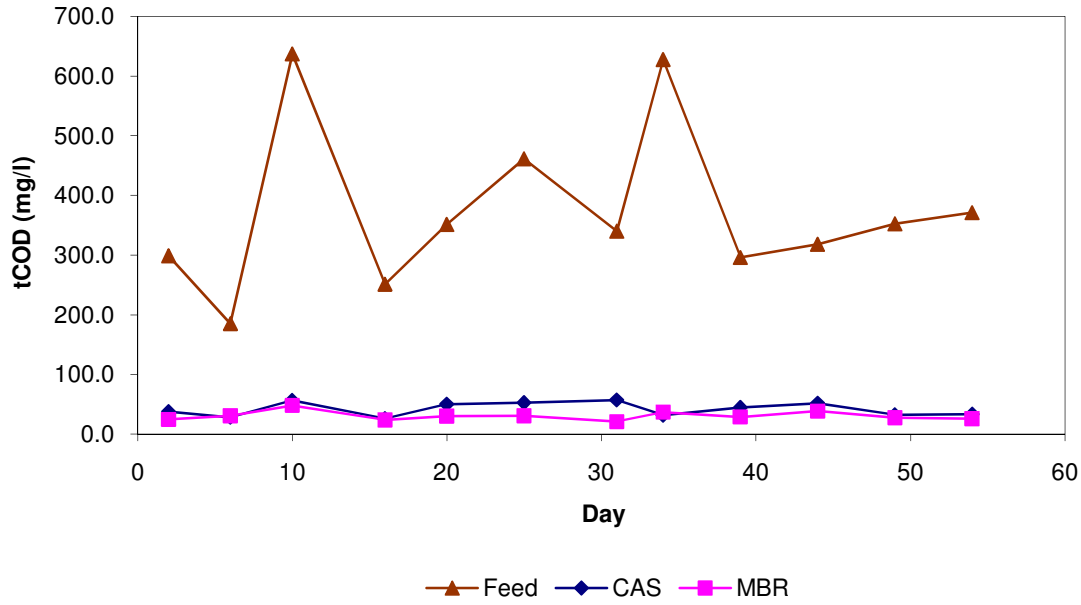


Figure 4.16 tCOD concentrations of UASB feed, CAS and MBR effluents with 0% sewage addition.

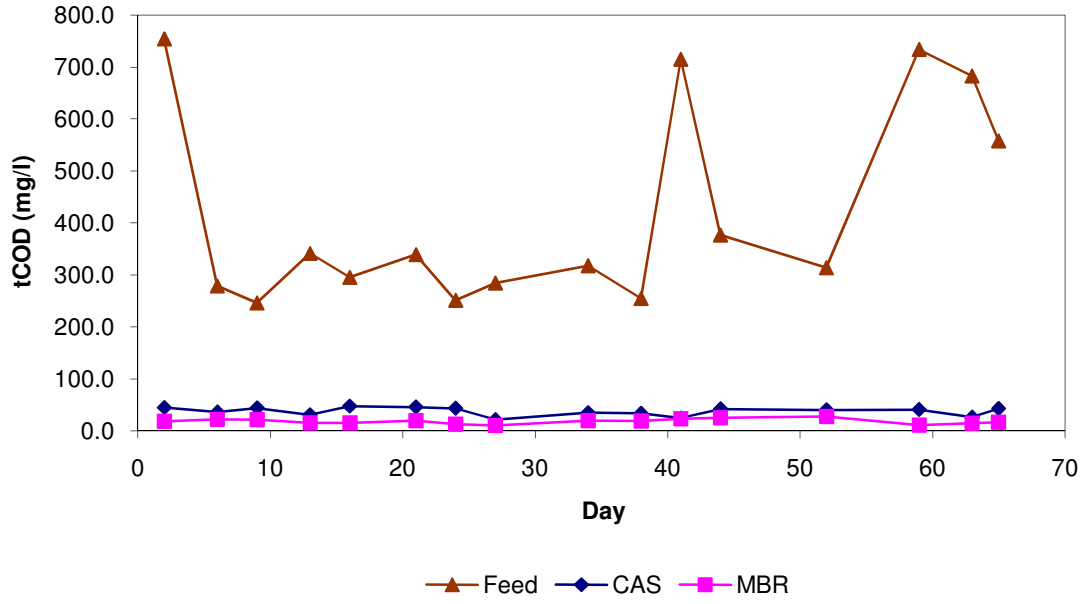


Figure 4.17 tCOD concentrations of AF feed, CAS and MBR effluents with 0% sewage addition.

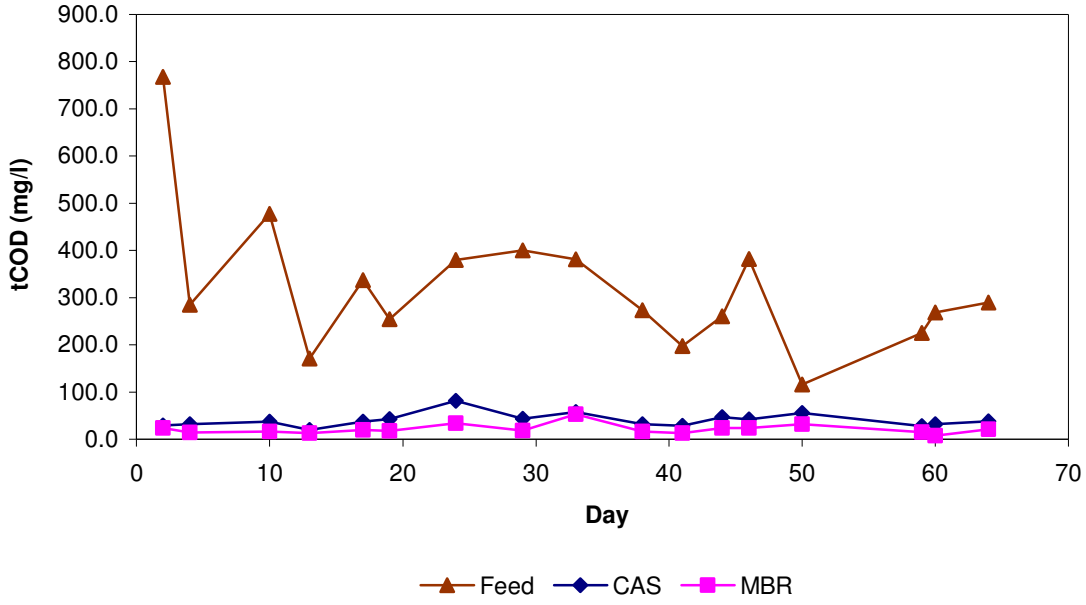


Figure 4.18 tCOD concentrations of anSBR feed, CAS and MBR effluents with 0% sewage addition.

Table 4.8 Summary of tCOD removal performances for the post-treatments with 0% sewage addition.

		Post-treatment for	
	UASB	AF	anSBR
tCOD (mg/l)			
Feed	374.1	421.5	321.5
CAS (effluent)	41.6	37.6	40.1
MBR (effluent)	30.6	18.7	21.2
Removal efficiency (%)			
CAS	88.1	89.4	85.3
MBR	91.2	94.7	92.3
F/M (mg COD/mg MLVSS)			
CAS	0.047	0.064	0.047
MBR	0.043	0.045	0.029

4.2.1.2 Nitrogen removal

Table 4.10 summarizes the average TN removal efficiency and effluent quality of CAS and MBR. The nitrogen removal efficiency had improved tremendously from 20% to approximately 70% with the introduction of anoxic tanks for post-treatment systems treating UASB and AF effluents. Average NO_3^--N concentrations in the post-treatment effluents were consistently less than 20 mg/l.

However, similar performance was not achieved in post-treatment for anSBR. The TN removal efficiencies of both CAS and MBR for anSBR effluents were only about 50%. It was found that the average tCOD of anSBR effluent was 291.7 mg/l, which was lower than UASB (tCOD = 374.4 mg/l) and AF (tCOD = 482.1 mg/l). Hence, the poorer performance could be attributed to the lower organic concentrations in the influent.

Both UASB and anSBR were having sludge removal to control SRT and accumulation of SS within the system; however sludge removal was not possible in AF, due to the internal structure of AF. As such, SS accumulated in the system and occasionally sludge washout took place when SS accumulation reached the limit and it resulted in higher tCOD to the post-treatment.

Table 4.9 Average TN removal efficiency and effluent quality of CAS and MBR with 0% sewage addition

(in mg/l)	Anaerobic pre-treatment processes		
	UASB	AF	AnSBR
Influent			
TN	54.2 ± 5.7	49.9 ± 10.8	52.8 ± 4.4
NH_4^+-N	39.5 ± 12.2	35.5 ± 5.6	39.1 ± 7.1
NO_2^--N	N.D.	N.D.	N.D.
NO_3^--N	N.D.	N.D.	N.D.
CAS			
TN	13.1 ± 1.8	13.1 ± 4.6	28.4 ± 3.2
NH_4^+-N	0.4 ± 0.9	0.9 ± 0.8	1.2 ± 0.8
NO_2^--N	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
NO_3^--N	8.5 ± 0.6	9.6 ± 4.4	22.6 ± 7.5
TN removal efficiency (%)	75.9 ± 2.0	71.5 ± 15.2	45.9 ± 6.9
MBR			
TN	17.2 ± 4.5	12.3 ± 4.0	26.7 ± 4.8
NH_4^+-N	N.D.	0.1 ± 0.4	0.7 ± 0.4
NO_2^--N	0.7 ± 0.9	0.1 ± 0.1	N.D.
NO_3^--N	12.3 ± 4.1	10.6 ± 7.2	23.8 ± 5.4
TN removal efficiency (%)	68.5 ± 7.3	74.1 ± 11.0	49.1 ± 10.1

N.D. – non detectable

4.2.2 75% anaerobic effluents with 25% raw sewage

25% of raw sewage was added to the anaerobic effluents based on the total flow rate to study the influence of organics on nitrogen removal. Both organic and nitrogen removal performances were monitored.

4.2.2.1 Organic removal

Figures 4.19, 4.20 and 4.21 show the graphs of tCOD concentrations for the different post-treatment systems operated with 75% anaerobic effluents and 25% raw sewage. The average values of tCOD are summarized in Table 4.11. The addition of raw sewage had caused increases in tCOD concentration to the post-treatment systems. All the post-treatments were able to achieve high removal efficiencies as shown in Table 4.11 with the increase in tCOD. Consistent tCOD concentrations below 50 mg/l were achieved by all the post-treatment systems.

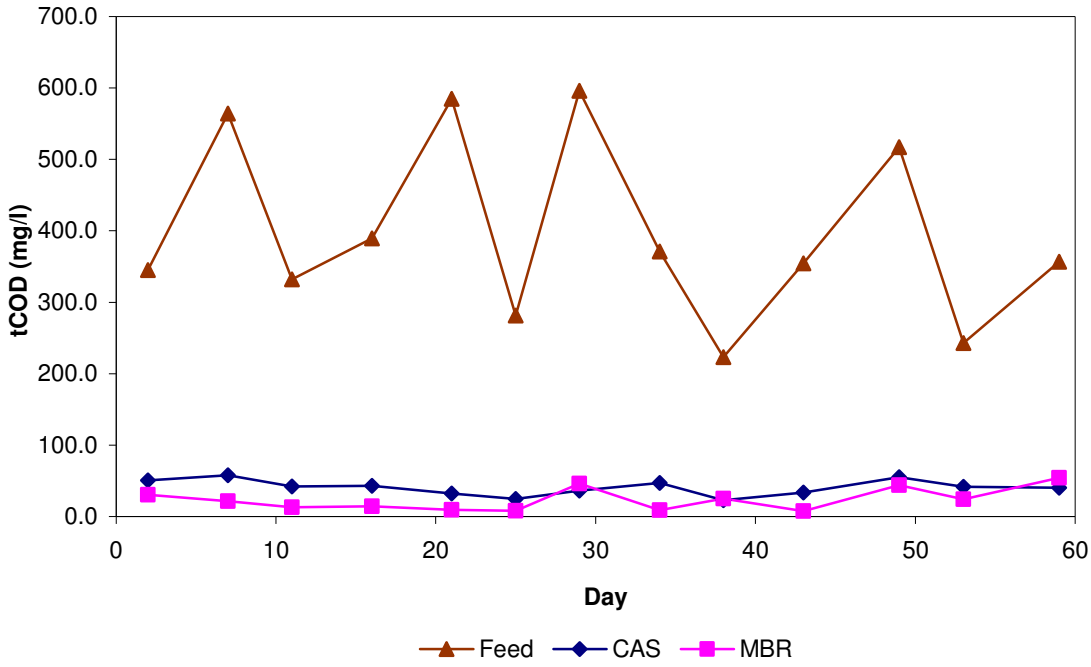


Figure 4.19 tCOD concentrations of UASB feed, CAS and MBR effluents with 25% sewage addition.

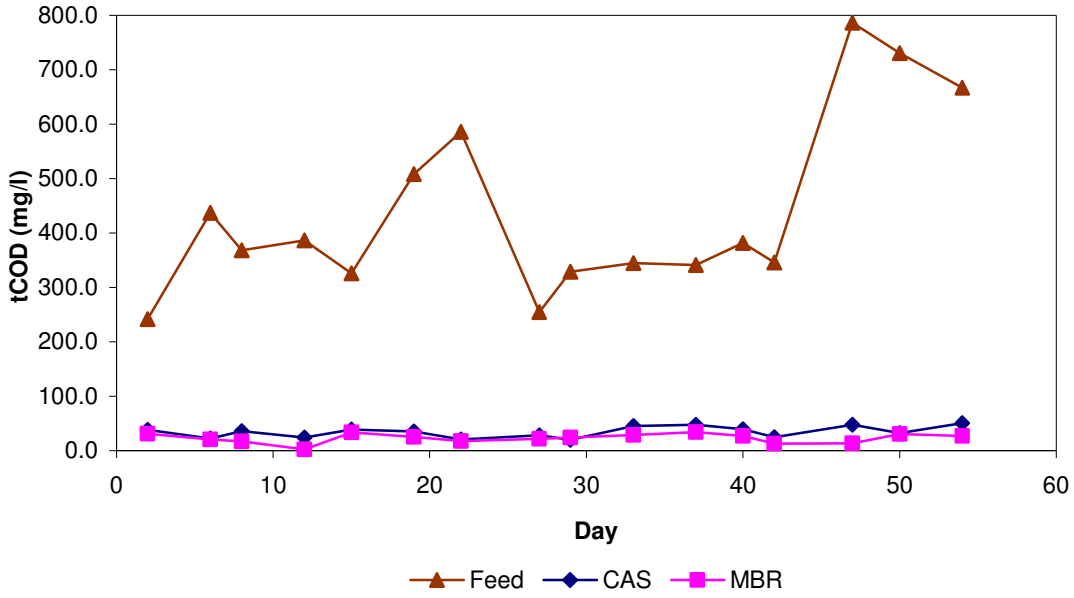


Figure 4.20 tCOD concentrations of AF feed, CAS and MBR effluents with 25% sewage addition.

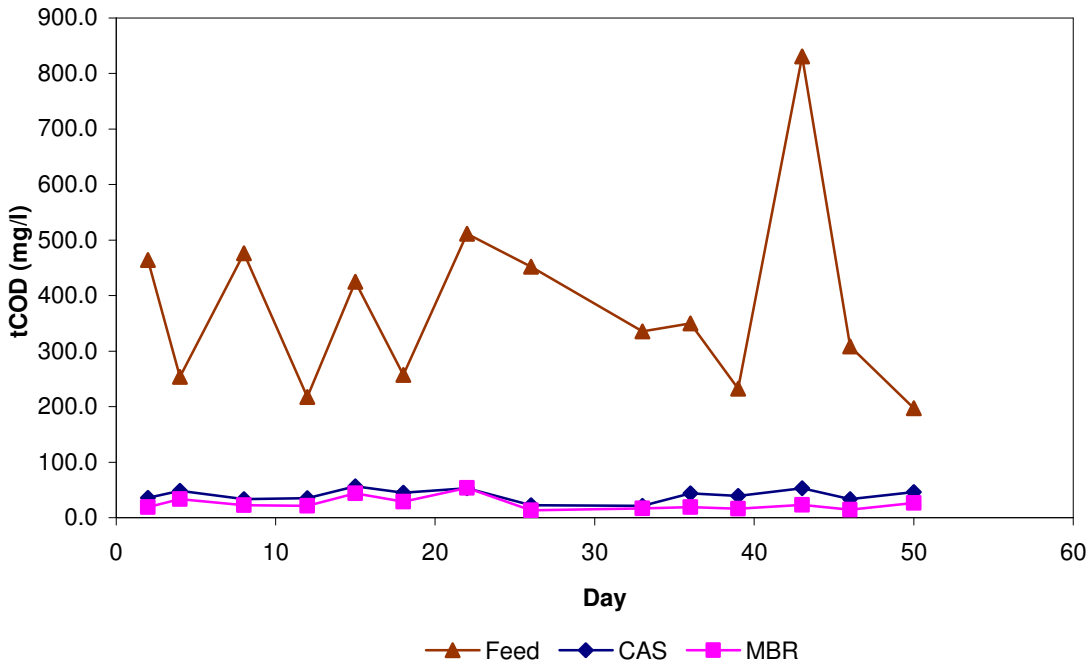


Figure 4.21 tCOD concentrations of anSBR feed, CAS and MBR effluents with 25% sewage addition.

Table 4.10 Summary of tCOD removal performances for the post-treatment systems with 25% sewage addition

	Post-treatment for		
	UASB	AF	anSBR
tCOD (mg/l)			
Feed	396.7	439.6	379.4
CAS (effluent)	40.5	34.5	40.5
MBR (effluent)	23.6	22.9	25.1
Removal efficiency (%)			
CAS	87.1	91.3	87.7
MBR	92.7	94.0	92.5
F/M (mg COD/mg MLVSS)			
CAS	0.055	0.065	0.051
MBR	0.043	0.046	0.035

4.2.2.2 Nitrogen removal

Table 4.12 summarizes the average TN removal efficiency and effluent quality of CASs and MBRs after the addition of 25% of raw sewage. TN removal efficiencies for post-treatments of UASB and AF were similar to the operating condition when 0% of raw sewage was added. However, TN removal efficiencies of post-treatment for anSBR effluent had improved tremendously by 16 and 19% for CAS and MBR, respectively. This was due to the increase amount of carbon compared to earlier case without raw water addition.

Competition of substrates among different microorganisms populations in a biological nutrient removal (BNR) system causes a decrease in effectiveness of nitrification and denitrification processes. Besides nitrification, denitrification was also influenced by the influent COD/N ratio. Carrera *et al.* (2004) reported that 39% of organic influent matter proved to be consumed by oxidation in a BNR system. That means that the COD/N ratio needed to denitrify all influent nitrogen with ethanol was 7.1 g COD / g N.

The removal efficiencies were comparable to that of post-treatment of UASB and AF effluents. The average NO_3^--N concentrations for all the three post-treatments were consistently below 20 mg/l. It had shown that the increase of COD in the influent did improve the nitrogen removal performance, although it was not very significant for post-treatment of UASB and AF effluents.

Table 4.11 Average TN removal efficiency and effluent quality of CAS and MBR with 25% sewage addition.

(in mg/l)	Anaerobic pre-treatment processes		
	UASB	AF	AnSBR
Influent			
TN	51.3 ± 9.7	49.7 ± 11.8	56.2 ± 8.2
NH_4^+-N	37.8 ± 9.3	40.2 ± 4.2	41.0 ± 6.4
NO_2^--N	N.D.	N.D.	N.D.
NO_3^--N	N.D.	N.D.	0.2 ± 0.6
CAS			
TN	12.6 ± 5.3	12.4 ± 1.3	20.8 ± 6.2
NH_4^+-N	N.D.	N.D.	1.4 ± 0.5
NO_2^--N	0.4 ± 0.4	N.D.	0.1 ± 0.1
NO_3^--N	6.5 ± 1.3	9.6 ± 1.3	15.6 ± 7.2
TN removal efficiency (%)	75.4 ± 8.3	69.0 ± 18.3	62.1 ± 12.9
MBR			
TN	13.0 ± 6.1	11.3 ± 2.1	17.6 ± 7.1
NH_4^+-N	0.4 ± 0.6	N.D.	1.2 ± 0.6
NO_2^--N	0.3 ± 0.2	0.2 ± 0.2	0.1 ± 0.1
NO_3^--N	10.0 ± 5.6	8.6 ± 1.7	8.8 ± 4.5
TN removal efficiency (%)	74.4 ± 12.6	72.1 ± 15.6	68.0 ± 13.9

N.D. – non detectable

4.2.3 50% anaerobic effluents with 50% raw sewage

In the last operating condition, 50% of raw sewage was added to the anaerobic effluents based on the total flow rate to study the influence of organic on the nitrogen removal. As usual, both organic and nitrogen removal performances were monitored.

4.2.3.1 Organic removal

Figures 4.22, 4.23 and 4.24 show the graphs of tCOD concentrations for the different post-treatment systems operating with 50% of anaerobic effluents and 50% of raw sewage. The average values of tCOD are summarized in Table 4.12. The addition of raw sewage increased the average tCOD concentrations for the feed of the UASB, AF and anSBR post-treatment systems by approximately 30%, 20% and 30%, respectively. The post-treatments were still able to perform well to achieve consistent tCOD concentrations of below 50 mg/l for the treated effluents.

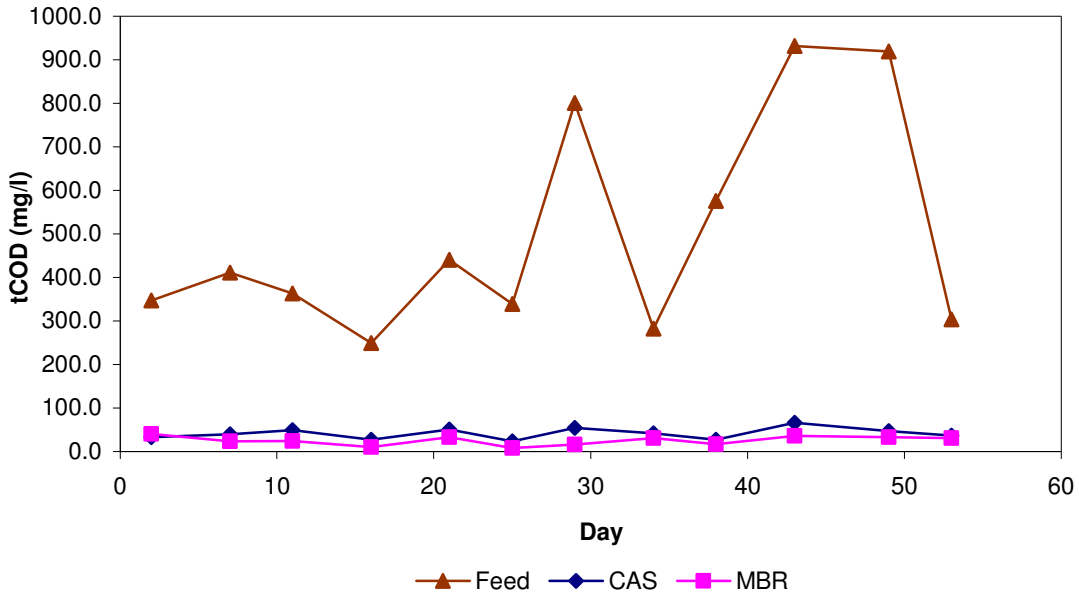


Figure 4.22 tCOD concentrations of UASB feed, CAS and MBR effluents with 50% sewage addition.

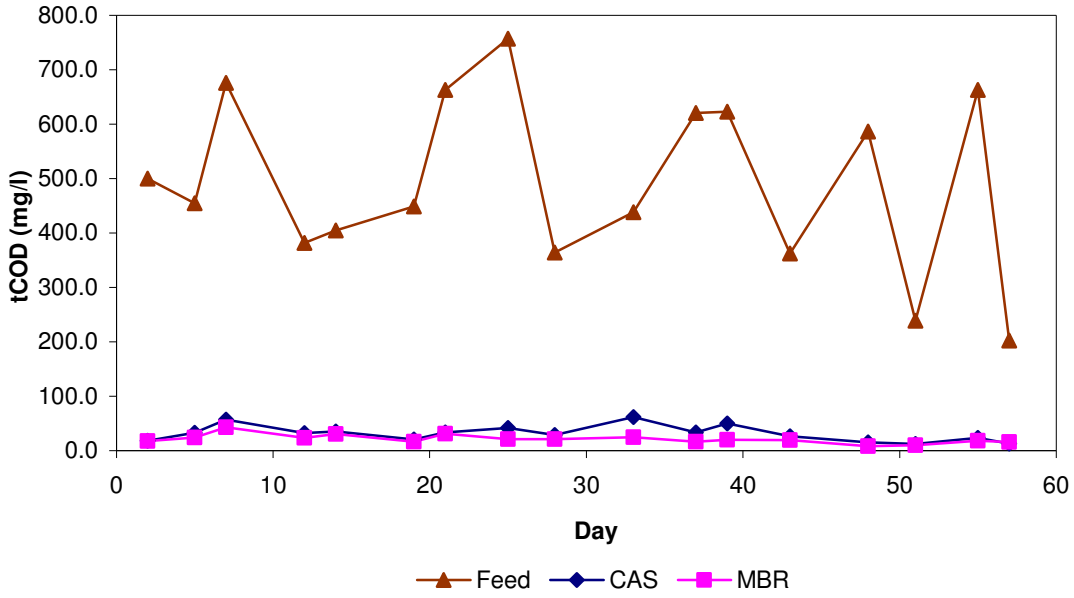


Figure 4.23 tCOD concentrations of AF feed, CAS and MBR effluents with 50% sewage addition.

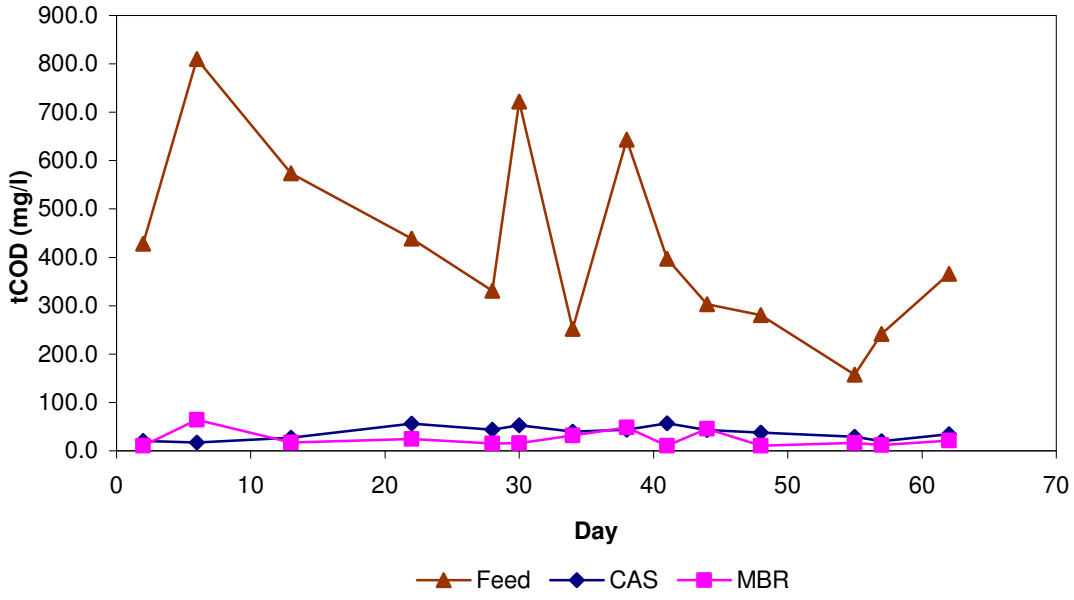


Figure 4.24 tCOD concentrations of anSBR feed, CAS and MBR effluents with 50% sewage addition.

Table 4.12 Summary of tCOD removal performances for the post-treatments with 50% of sewage addition.

	Post-treatment for		
	UASB	AF	anSBR
tCOD (mg/l)			
Feed	496.9	493.2	424.6
CAS (effluent)	41.5	31.5	37.3
MBR (effluent)	25.2	21.3	24.5
Removal efficiency (%)			
CAS	88.4	93.4	89.6
MBR	92.6	95.4	93.7
F/M (mg COD/mg MLVSS)			
CAS	0.084	0.096	0.069
MBR	0.045	0.054	0.039

4.2.3.2 Nitrogen removal

Table 4.14 shows the average TN removal efficiency and effluent quality of CAS and MBR after addition of 50% of raw sewage. More than 70% of TN removal efficiencies were achieved in all the post-treatments. Complete TN removal was difficult to achieve as aerobic reactor was the last biological reactor of the single-sludge reactor system, thus, a part of the generated NO_x^--N would flow out of the reactor system (Siebritz et. al., 1983). The removal efficiencies had improved by about 5%. However, nitrification performance deteriorated slightly with traces of NH_4^+-N found in the effluents of both the CAS and MBR.

Table 4.13 Average TN removal efficiency and effluent quality of CAS and MBR with 50% sewage addition.

(in mg/l)	Anaerobic pre-treatment processes		
	UASB	AF	AnSBR
Influent			
TN	62.4 ± 11.5	52.8± 10.0	45.8± 14.5
NH_4^+-N	45.0 ± 2.8	38.5 ± 8.0	37.4 ± 7.9
NO_2^--N	N.D.	N.D.	N.D.
NO_3^--N	N.D.	N.D.	N.D.
CAS			
TN	11.9 ± 3.6	12.9 ± 5.5	10.8 ± 4.7
NH_4^+-N	11.0 ± 4.9	3.2 ± 5.5	0.4 ± 1.5
NO_2^--N	0.7 ± 0.5	0.1 ± 0.1	0.1 ± 0.1
NO_3^--N	5.3 ± 2.4	7.3 ± 1.8	7.2 ± 2.8
TN removal efficiency (%)	85.5 ± 3.9	75.9 ± 7.7	76.8 ± 7.4
MBR			
TN	17.0 ± 2.9	12.2 ± 4.1	13.1 ± 5.7
NH_4^+-N	5.9 ± 8.0	1.7 ± 4.1	5.5 ± 10.3
NO_2^--N	0.8 ± 0.9	0.1 ± 0.2	0.1 ± 0.1
NO_3^--N	6.1 ± 3.4	8.1 ± 3.1	4.9 ± 3.1
TN removal efficiency (%)	79.3 ± 2.5	75.1 ± 6.5	70.4 ± 11.3

N.D. – non detectable

The deterioration of nitrification performance was possibly a consequence of the higher organic concentrations in the substrate due to the addition of 50% raw sewage. Table 4.15 summarises the average TOC concentrations of anaerobic effluent and raw sewage. Raw sewage was observed to contain a higher concentration of TOC as compared to anaerobic effluent. Hence, the readily biodegradable substrate encouraged the growth of heterotrophic bacteria. The typical growth yield of heterotrophic bacteria

and nitrifiers are 0.4 g VSS/g bCOD and 0.12 g VSS/g NH_4-N, respectively (Tchobanoglous and Burton, 2004).

Table 4.14 Average TOC concentrations of anaerobic effluent and raw sewage

(in mg/l)	Anaerobic pre-treatment processes		
	UASB	AF	AnSBR
Anaerobic effluent	23.5 ± 6.7	34.3 ± 13.8	25.8 ± 7.7
Raw sewage	35.2 ± 13.1	43.0 ± 15.5	42.4 ± 16.7

4.2.4 ΔCOD/ΔTN

Based on the transfer of one electron equivalent for dissimilative reduction of nitrate (i.e. denitrification), the reduction of 1 g of NO_3^--N requires the consumption of 2.86 g of COD. From Figures 4.25, 4.26 and 4.27, it was observed that the ΔCOD/ΔTN ratios within the 3 different post-treatment systems were all larger than 2.86. This implies that the substrate (COD) was not only consumed in denitrification, it was also consumed by either microbial synthesis or other aerobic heterotrophs. In addition, the ΔCOD/ΔTN decreased with the decrease in organic loading (F/M). This is mainly because the excess substrate was oxidized by the aerobic heterotrophs in the aerobic compartment which might affect the nitrification efficiency.

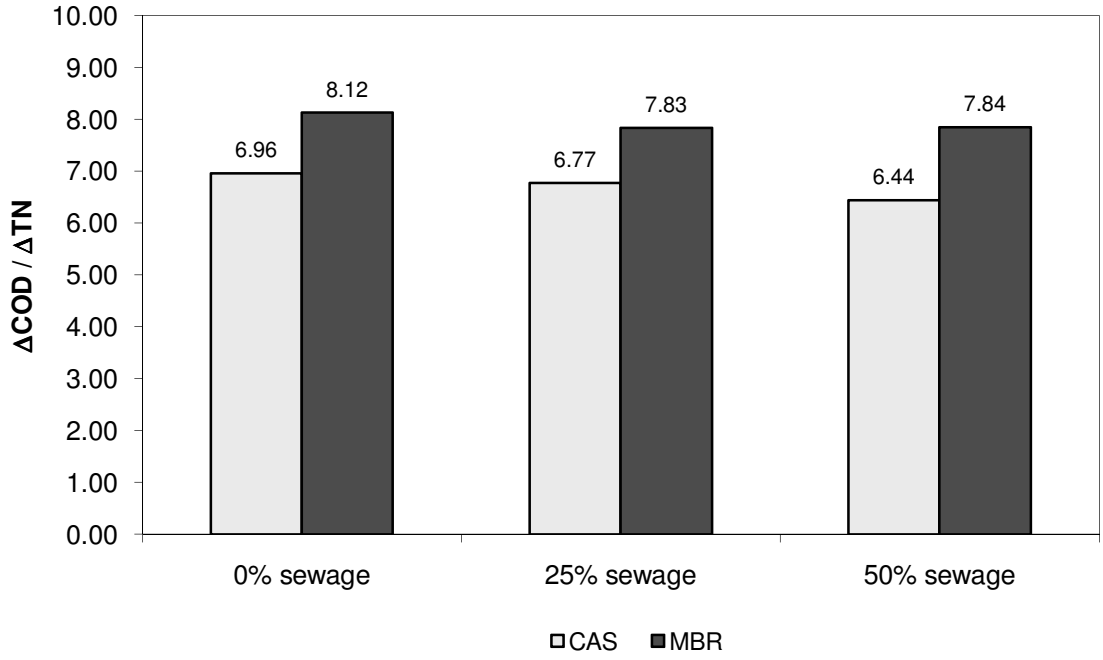


Figure 4.25 ΔCOD / ΔTN ratios for UASB post-treatment operating at different sewage addition

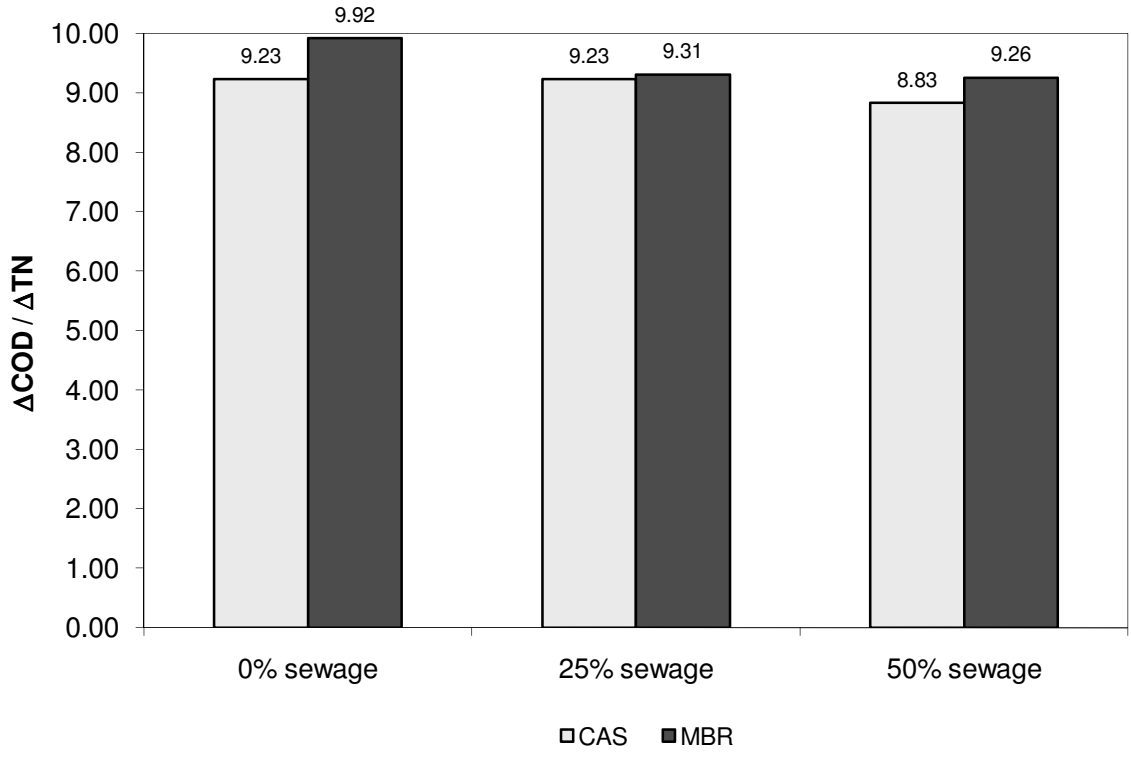


Figure 4.26 ΔCOD / ΔTN ratios for AF post-treatment operating at different sewage addition

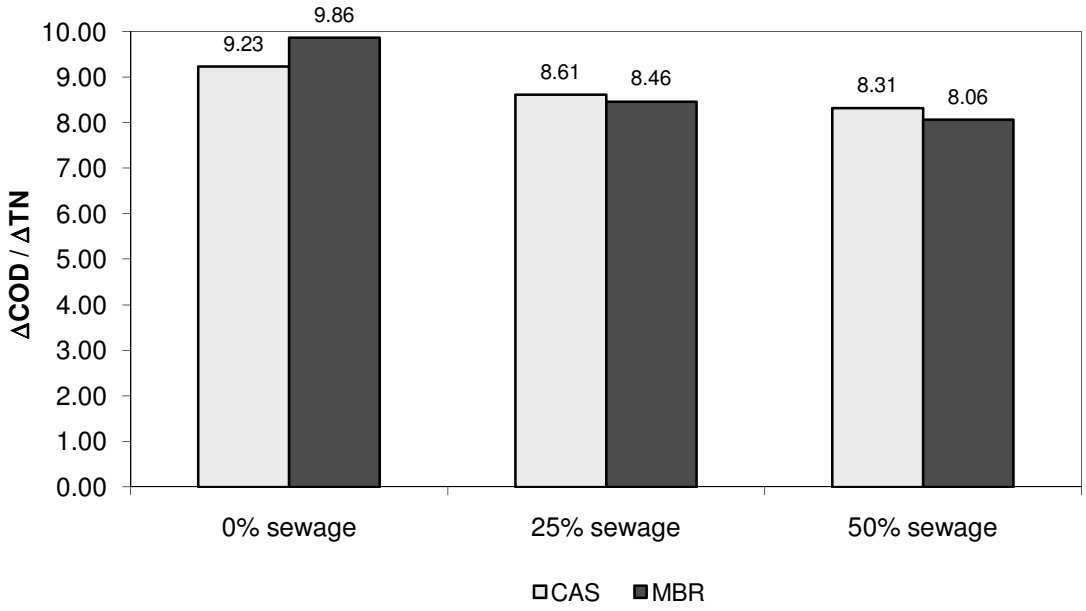


Figure 4.27 ΔCOD / ΔTN ratios for anSBR post-treatment operating at different sewage addition

4.3 Dissolved organic matter (DOM) size distribution

Knowledge about the distribution of molecular size fractions is very important for understanding the basic chemistry of DOM in water and its degradation and recycling. Figure 4.28 shows the results of molecular size distribution analysis for the effluents of UASB, AF and anSBR by applying batch UF. Data are shown as the percentage of DOC associated with discrete MW ranges. According to Logan and Jiang (1990), parallel processing of samples in batch mode is preferred since they can be run simultaneously and a smaller errors is observed in size distributions as opposed to series sample processing. Therefore, parallel sample processing was adopted in this study.

From Figure 4.28, it was observed that major parts of the molecules of DOM for the three different anaerobic effluents were distributed in the region smaller than 1 kDa with 57%, 60% and 53%, respectively. Findings by Barker *et. al.* (1998) found that majority of the material present in the effluents was in the low MW range (i.e. MW < 1kDa) while characterizing effluents from different anaerobic processes. In addition to VFA, the material is most likely to be residual feed, products of degradation of high MW material and smaller SMP (DeWalle and Chian, 1974; Rittmann *et. al*., 1987).

A relatively high percentage of DOM was distributed in the region from 1 to 10 kDa too. From Figure 4.28, it is clear that majority of the DOM (> 80%) were having the molecular weight of less than 10kDa. Only a small percentage of DOM was more than 10kDa. It could be due to the fact that the large MW molecules in the influent wastewater were metabolized into lower MW organics by microorganisms during the anaerobic process.

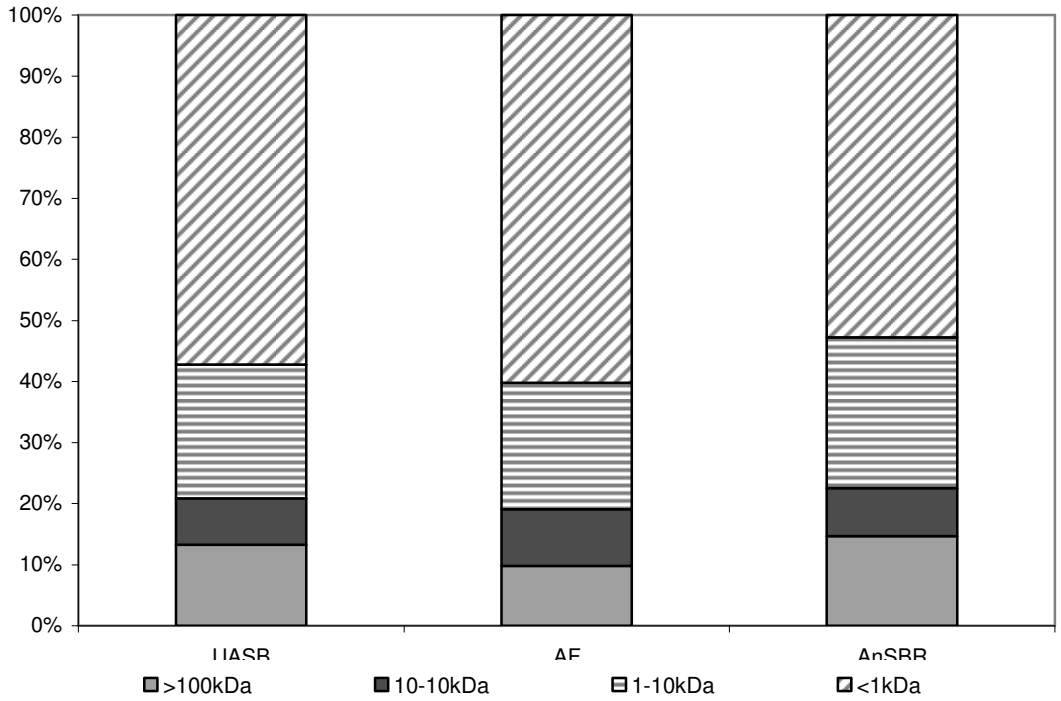


Figure 4.28 Molecular size distribution analysis for the effluents of UASB, AF and anSBR.

Figures 4.29, 4.30 and 4.31 show the results of molecular size distribution analysis for the post-treatment effluents for UASB, AF and anSBR, respectively, for various percentage of sewage addition. The percentage of MW > 10 kDA in the feed were observed to increase with the addition of sewage. As raw sewage had gone through minimal biological treatment, it would contain more higher MW organic compounds.

The percentages of smaller MW organic compounds increased after post-treatment. It showed that biodegradation of the larger MW organic compounds took place, particularly in MBR which could achieve higher biodegradation. The reasons for the higher biodegradation in MBR could be attributed to the higher biomass concentration and the longer SRT that was used.

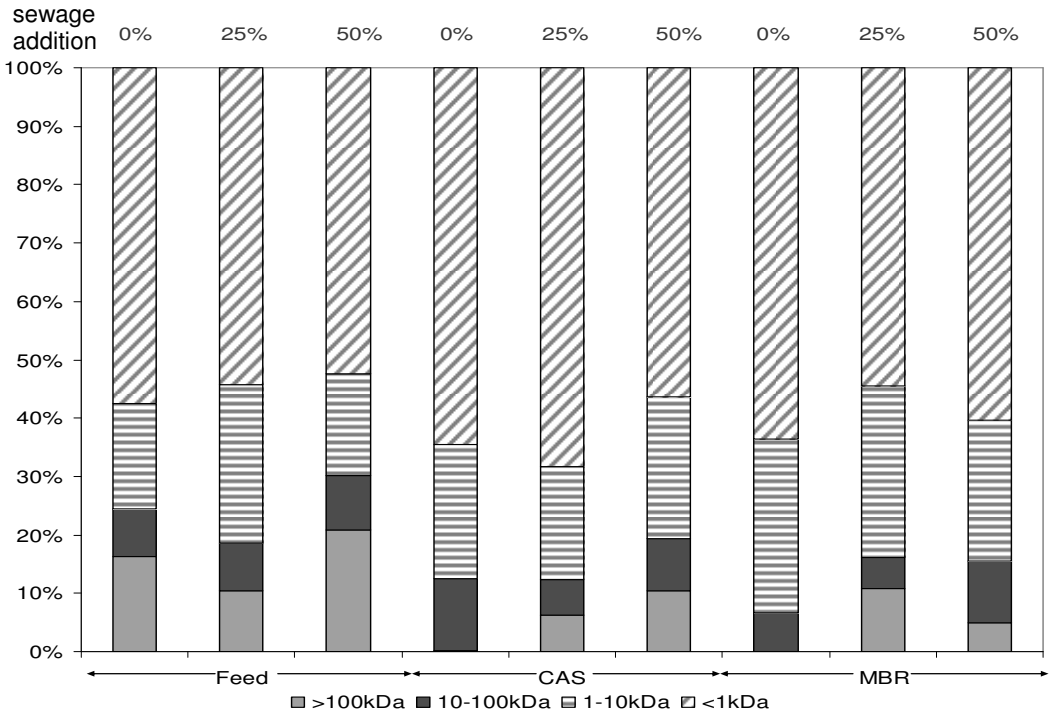


Figure 4.29 Molecular size distribution analysis for the UASB effluents (feed) and aerobic post-treatment effluents of UASB.

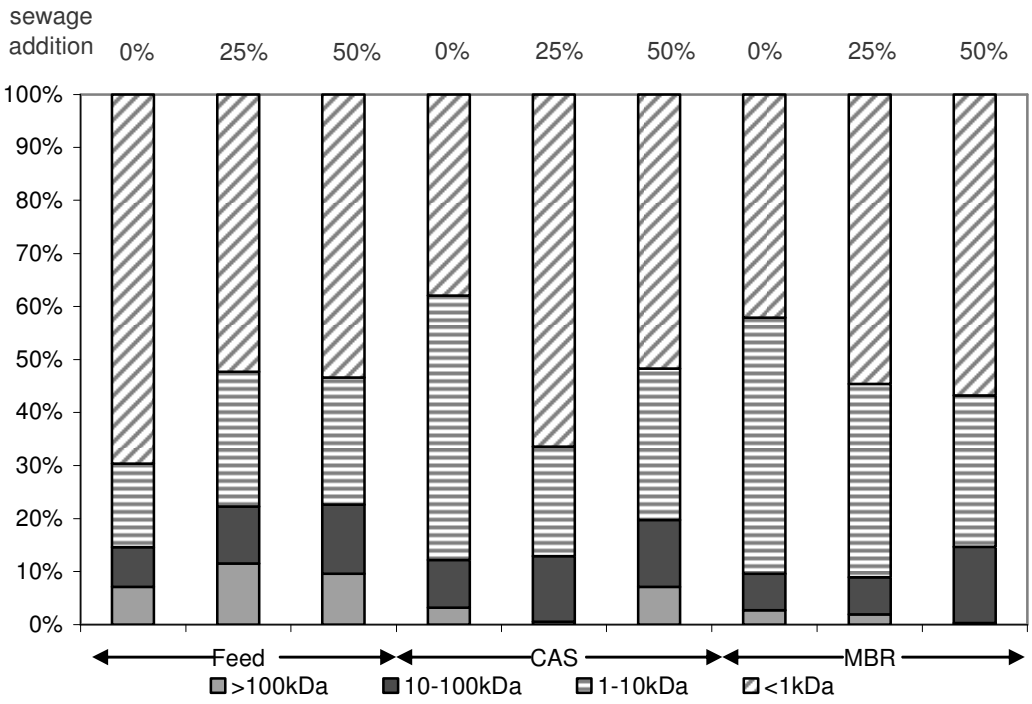


Figure 4.30 Molecular size distribution analysis for the AF effluents (feed) and the aerobic post-treatment effluents of AF

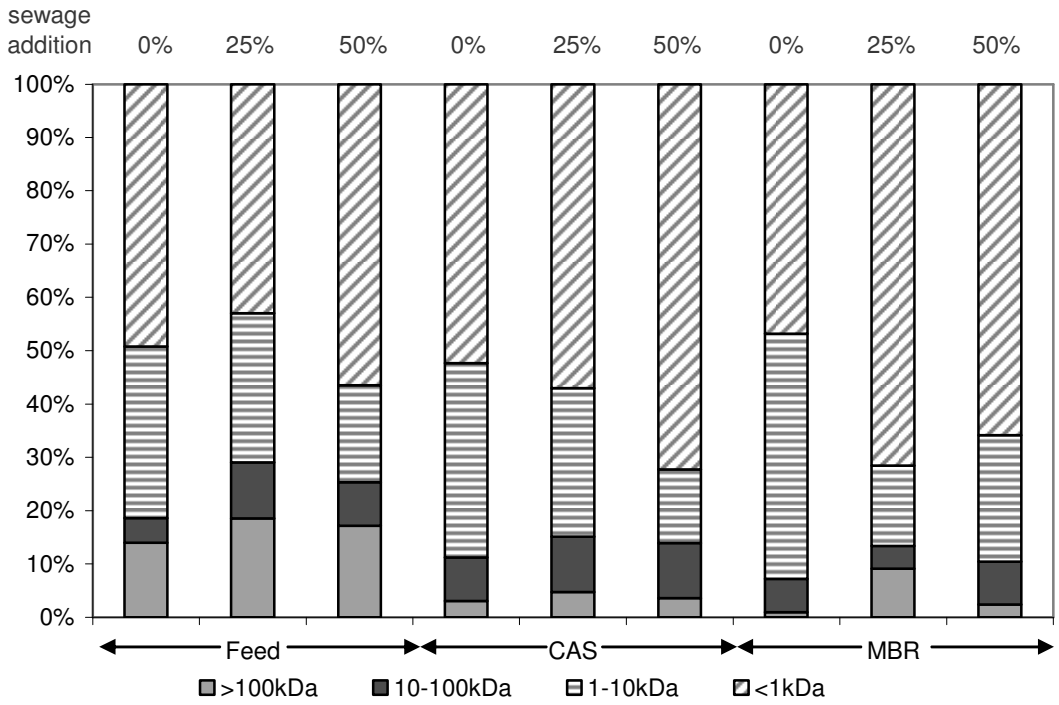


Figure 4.31 Molecular size distribution analysis for the anSBR effluents (feed) and the aerobic post-treatment effluents of anSBR

It was observed that in general, bigger percentage of smaller MW organic compounds were found in MBR effluents than CAS effluents. The differences may correspond to the organic removal mechanisms in these two processes. The bacterial metabolism plays a major role in the removal of organic substances in both CAS and MBR processes, which contributes to the similar transformation of MW of organic matters. However, the MBR system could enhance the organic removal efficiency by employing the membrane and longer SRT compared to the CAS process (Huang *et al.*, 2007). The retention of macromolecular by the membrane, as indicated by the difference percentage of MW molecules between the two processes, facilitates the removal of organic matters (microorganisms could degrade them, to some extent, at longer SRT) and improves the effluent water quality of MBRs.

However, caution should be exercised when comparing results from different studies since different process controls, cultures and techniques are used. In addition, MW determination by UF has a number of limitations. The diffusion and advective transport of organics through UF membranes is influenced by a variety of factors, including membrane pore size distribution, water temperature, cell pressure, solution pH and ionic strength as well as molecular size, shape and affinity for the different membrane materials (Logan and Jiang, 1990).

4.4 T-RFLP analysis

In this study, 16S rRNA gene was used to target the microbial community in both CAS and MBR mixed liquors treating anaerobic effluent from AF at different operating conditions. This was aimed to study the dynamics of the microbial community in the different post-treatment systems. The PCR amplifications of 16S rRNA genes were performed with both archaea and bacteria specific primers. Figures 4.32 and 4.33 show the T-RFLP fingerprint profiles of CAS and MBR operating at 8 h HRT using RsaI restriction enzyme. The samples were taken from the aeration tanks of both CAS and MBR after steady states were reached.

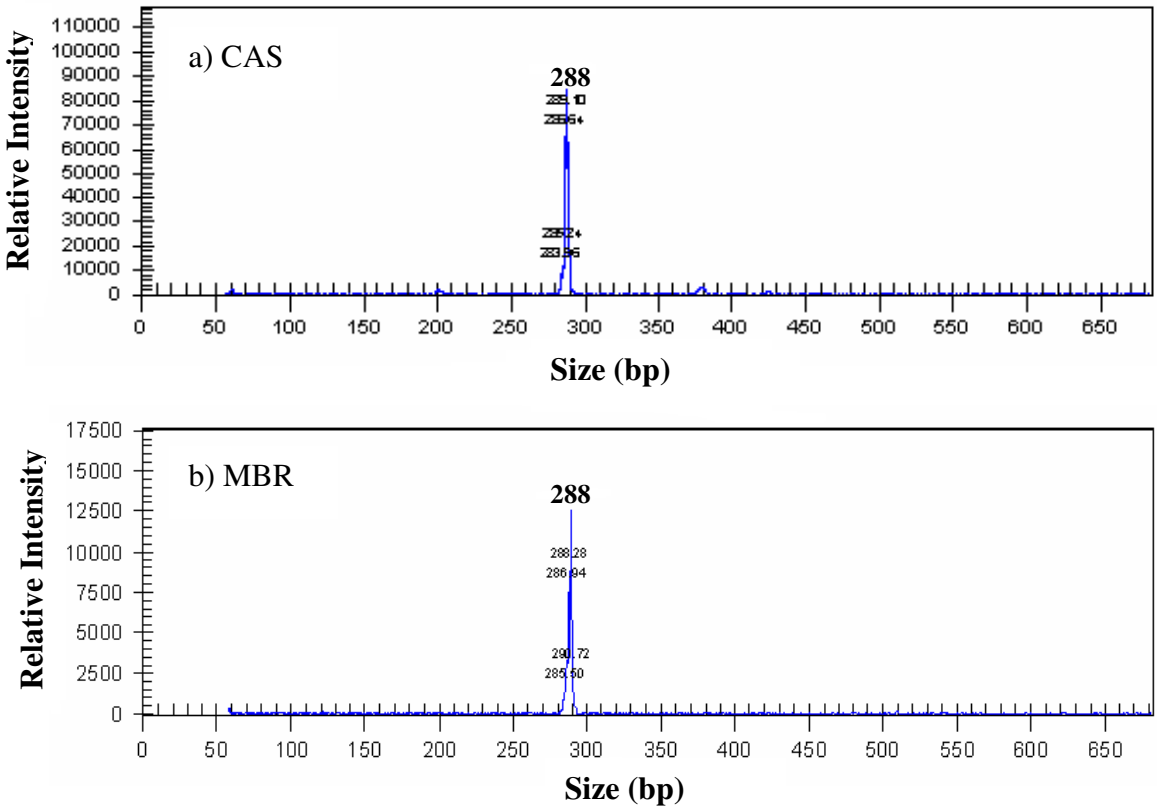


Figure 4.32 T-RFLP fingerprint profiles of CAS and MBR at 8 h HRT using archaea-specific primers and RspI enzyme.

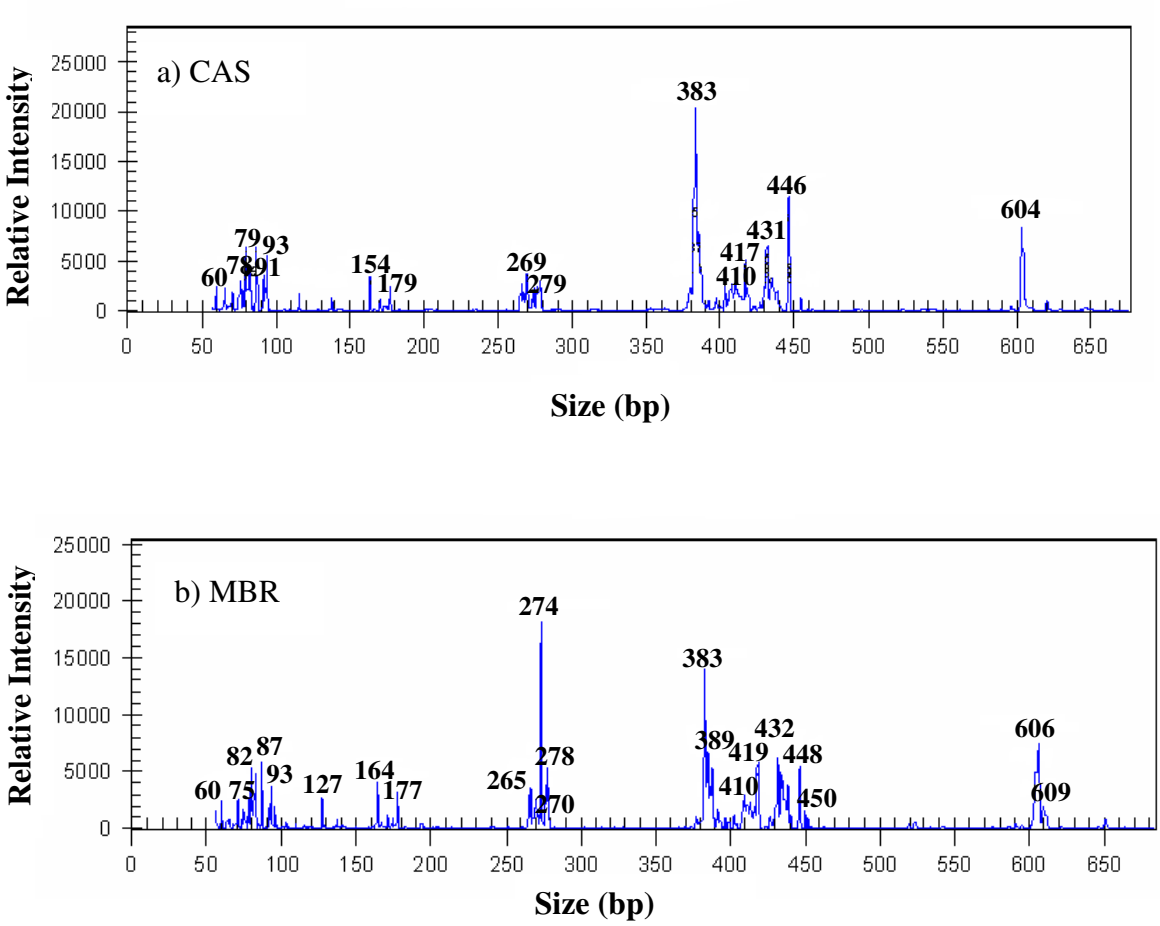


Figure 4.33 T-RFLP fingerprint profiles of CAS and MBR at 8 h HRT using bacteria-specific primers and RspI enzyme.

The prominent peaks were identified and the correspondence bp values are shown in the figures. Only one prominent peak was observed for T-RFLP fingerprint profiles using archaea-specific primer for both CAS and MBR, as shown in Figures 4.32a and 4.32b. It was not surprising as both systems were aerobic, thus favored the dominant of bacteria. On the other hand, multiple peaks were found in Figures 4.33a and 4.33b when bacteria-specific primers were used. The species from the different systems were similar as both systems were fed with the same anaerobic effluent from AF. However, the dominant species were different.

Besides samples from phase 1 of the study, T-RFLP analysis was also applied to the samples from phase 2 of the study. In addition to aeration tanks, samples from anoxic tanks were also taken. Three different feed compositions for the post-treatment systems were studied in phase 2. The first type of feed consisted of only anaerobic effluents. 25% sewage was added to the post-treatment as the second type of feed and sewage was finally increased to 50% as third type of feed.

Figures 4.34 and 4.35 show the T-RFLP fingerprint profiles for both aeration and anoxic tanks of CAS and MBR using RspI restriction enzyme with archaea and bacteria-specific primers, respectively.

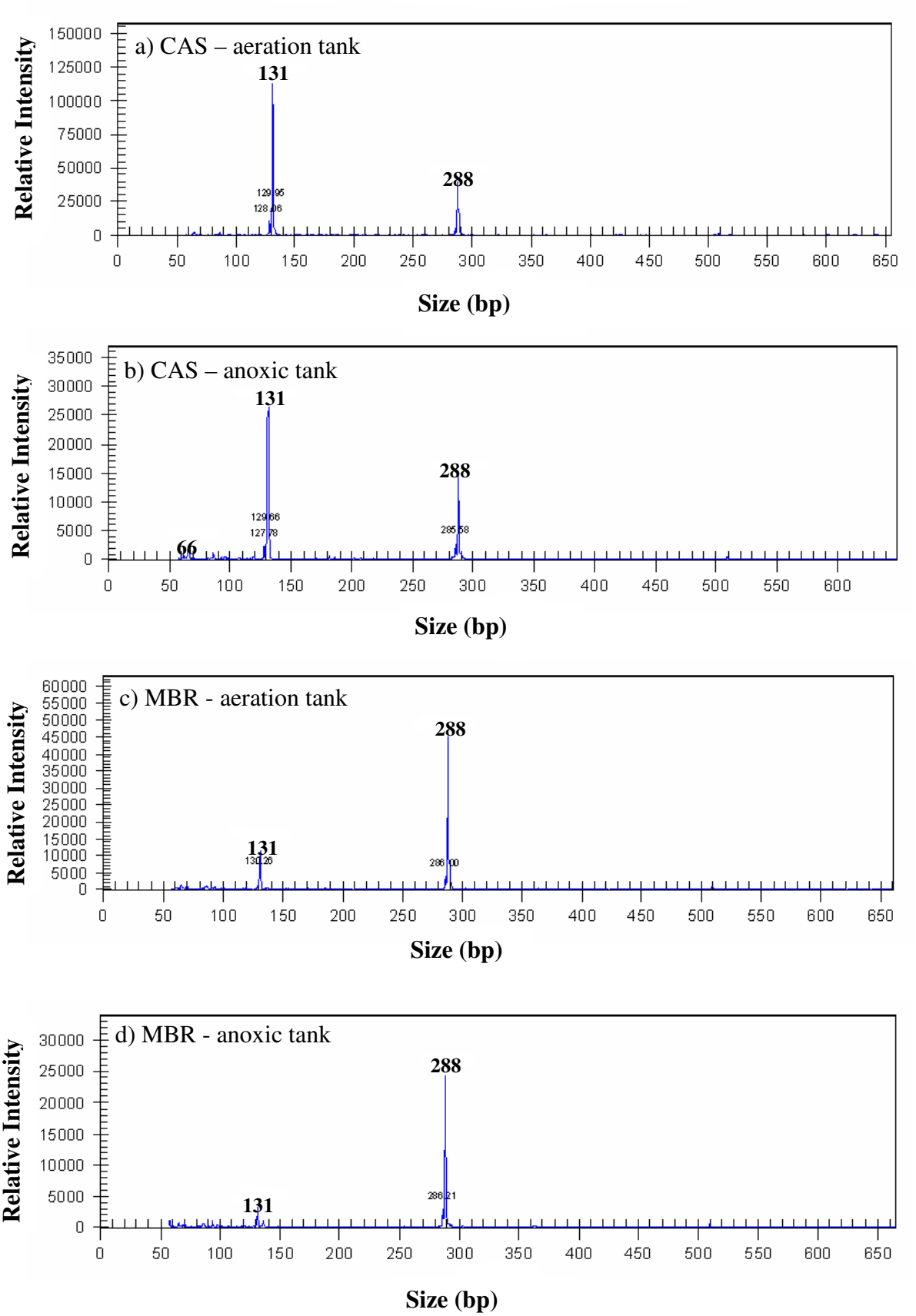


Figure 4.34 T-RFLP fingerprint profiles of aeration and anoxic tanks of CAS and MBR at 8-h HRT using archaea-specific primers and RspI enzyme.

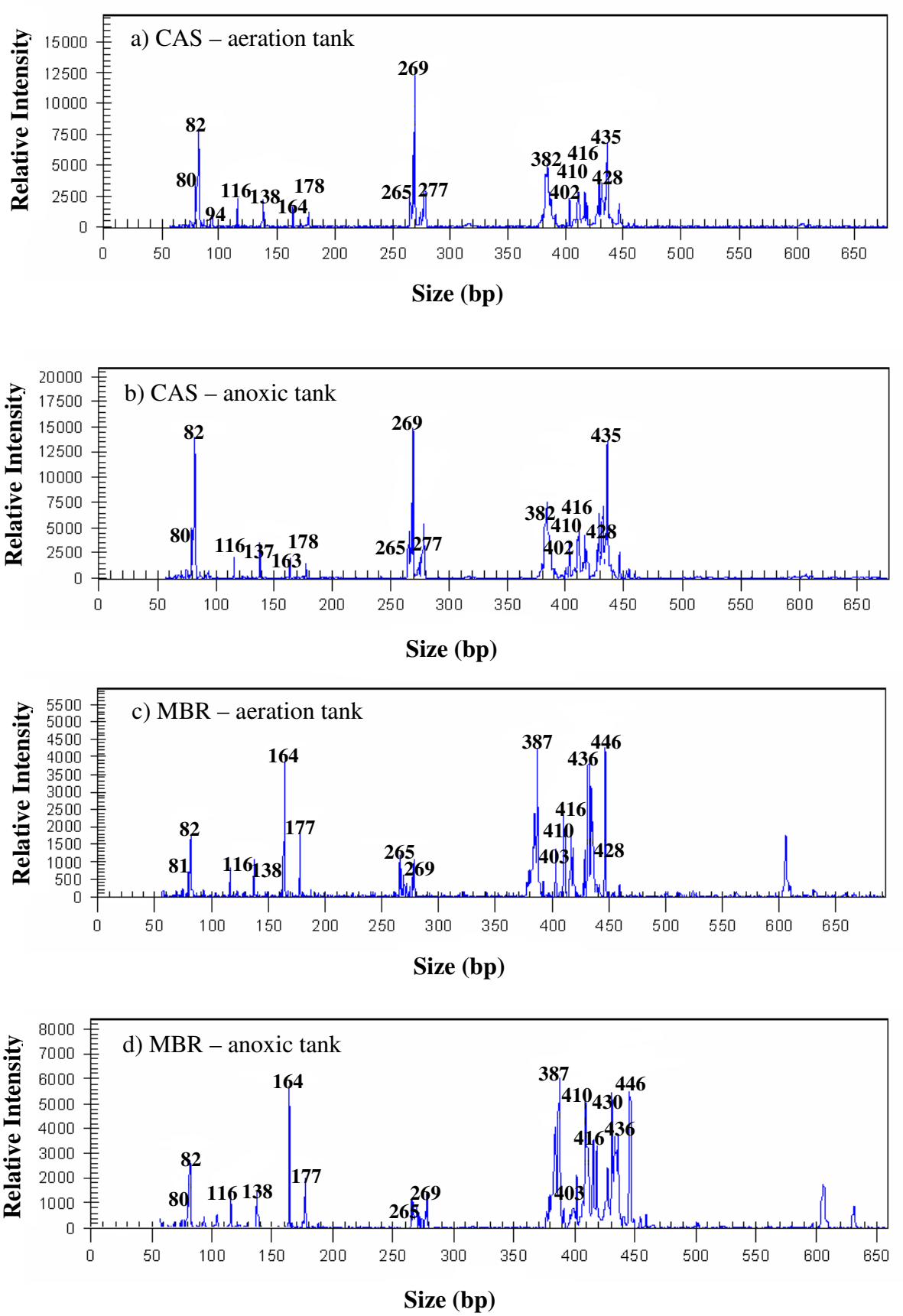


Figure 4.35 T-RFLP fingerprint profiles of aeration and anoxic tanks of CAS and MBR at 8-h HRT using bacteria-specific primers and RspI enzyme.

As compared to phase 1 of the study where only aeration tanks were used, additional peak at 131 bp was observed in the T-RFLP fingerprint profiles when archaea-specific primer was used. This should be due to the presence of anoxic tanks in the post-treatments where species of archaea with minimal reliance of oxygen thrived. The dominated species in CAS and MBR was found to be different. The dominated species in CAS was peaked at 131 bp while 288 bp was found to dominate MBR.

As there was recirculation of sludge from aeration to anoxic tank, the species in both tanks within the same system were expected to be similar. It was confirmed by the T-RFLP fingerprint profiles where similar species with similar intensity were found.

Despite of the similar bacteria species within the two different systems, there were distinct domineering species. From Figures 4.35a and 4.35b, domineering species in CAS was at 269 bp. On the other hand, Figures 4.35c and 4.35d revealed that the domineering species in MBR were very diverse. Multiple peaks were found between 387 and 446 bp. The peak at 164 bp was quite prominent too.

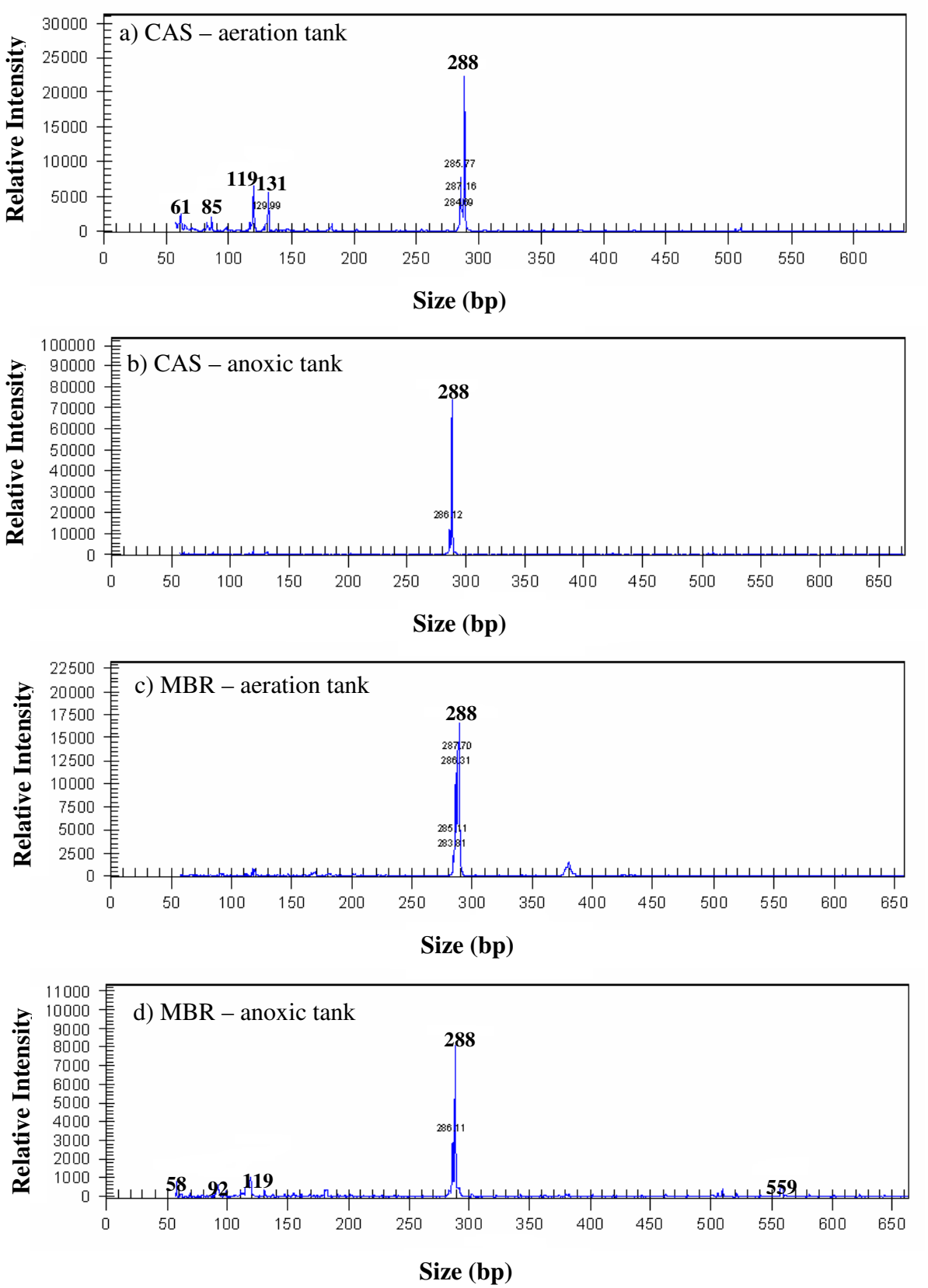


Figure 4.36 T-RFLP fingerprint profiles of aeration and anoxic tanks of CAS and MBR at 8-h HRT using archaea-specific primers and RspI enzyme (25% sewage addition).

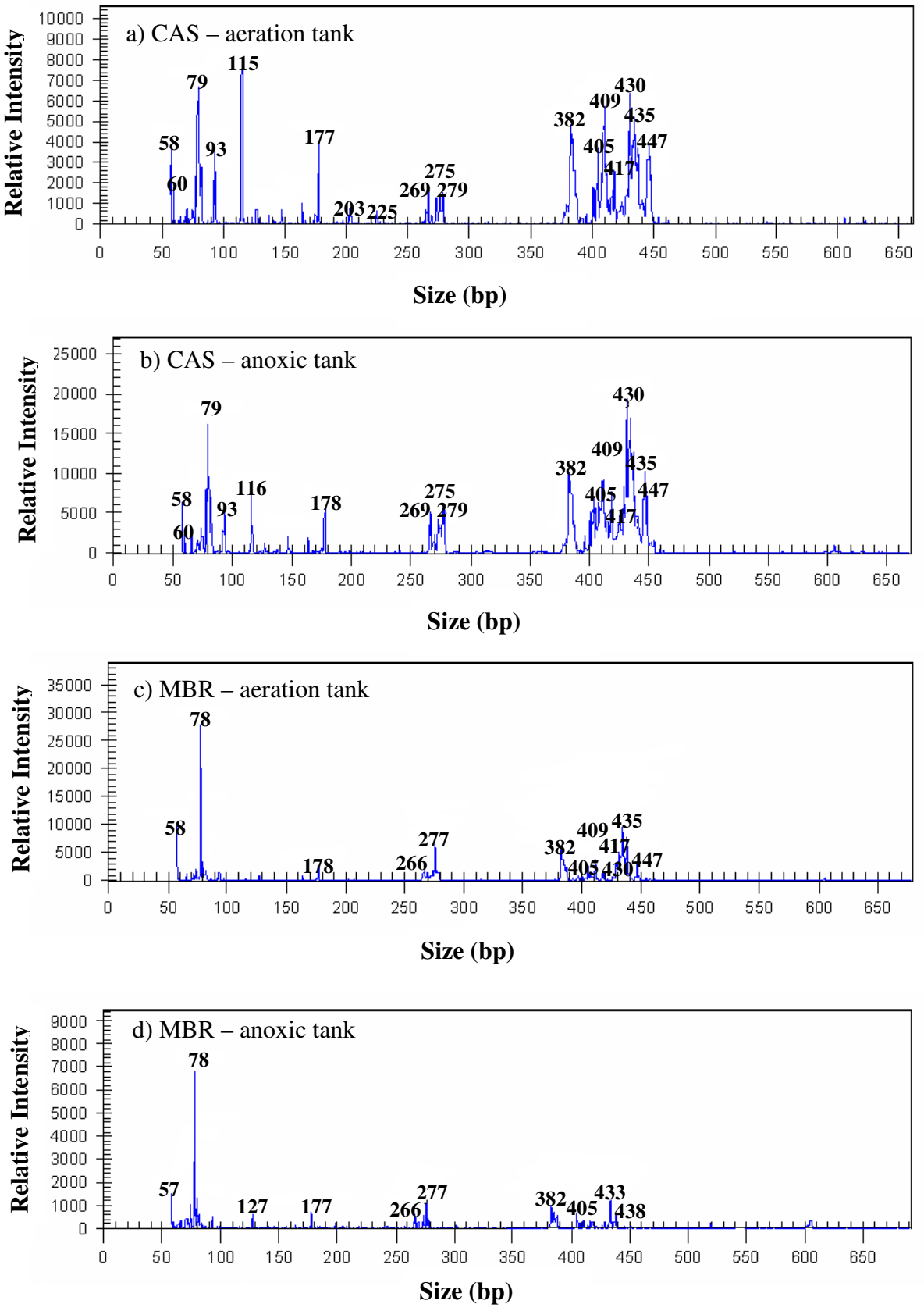


Figure 4.37 T-RFLP fingerprint profiles of aeration and anoxic tanks of CAS and MBR at 8-h HRT using bacteria-specific primers and RspI enzyme (25% sewage addition).

When 25% sewage was added to the post-treatment systems, only one dominant species at 288bp was found. The T-RFLP fingerprint profiles using archaea-specific primer are shown in Figure 4.36. It was also noted that the species with 131 bp which was found in the previous operating condition had diminished.

From Figure 4.37, the species in the CAS system were observed to be rather similar to the previous operating condition. The domineering species at 164 bp reduced while that at 79 bp increased. The shift in bp should be due to the presence of more easily biodegradable substances in the sewage. Similar increase in 79 bp was also observed in MBR as shown in Figures 4.6c and 4.6d.

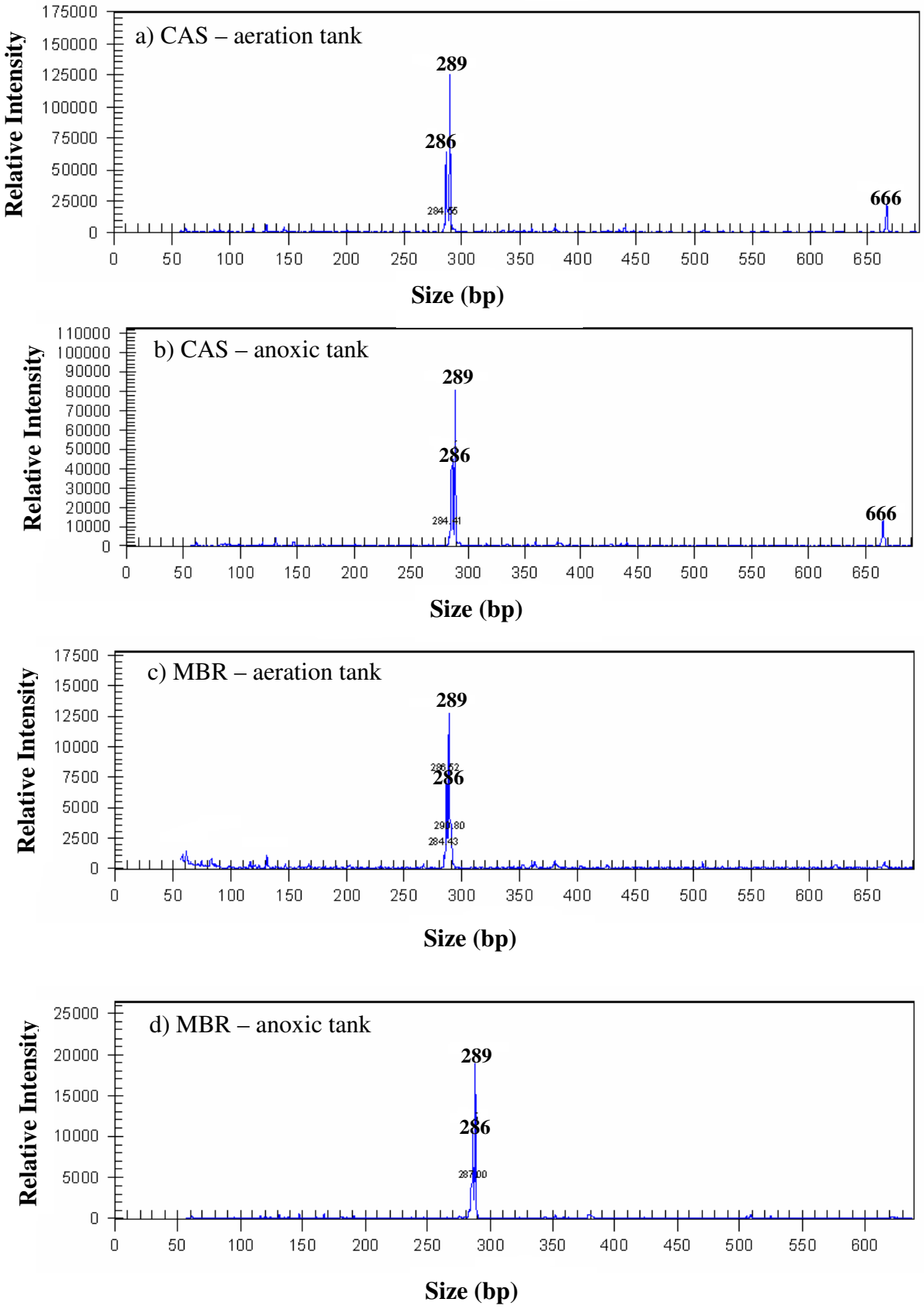


Figure 4.38 T-RFLP fingerprint profiles of aeration and anoxic tanks of CAS and MBR at 8-h HRT using archaea-specific primers and RspI enzyme (50% sewage addition).

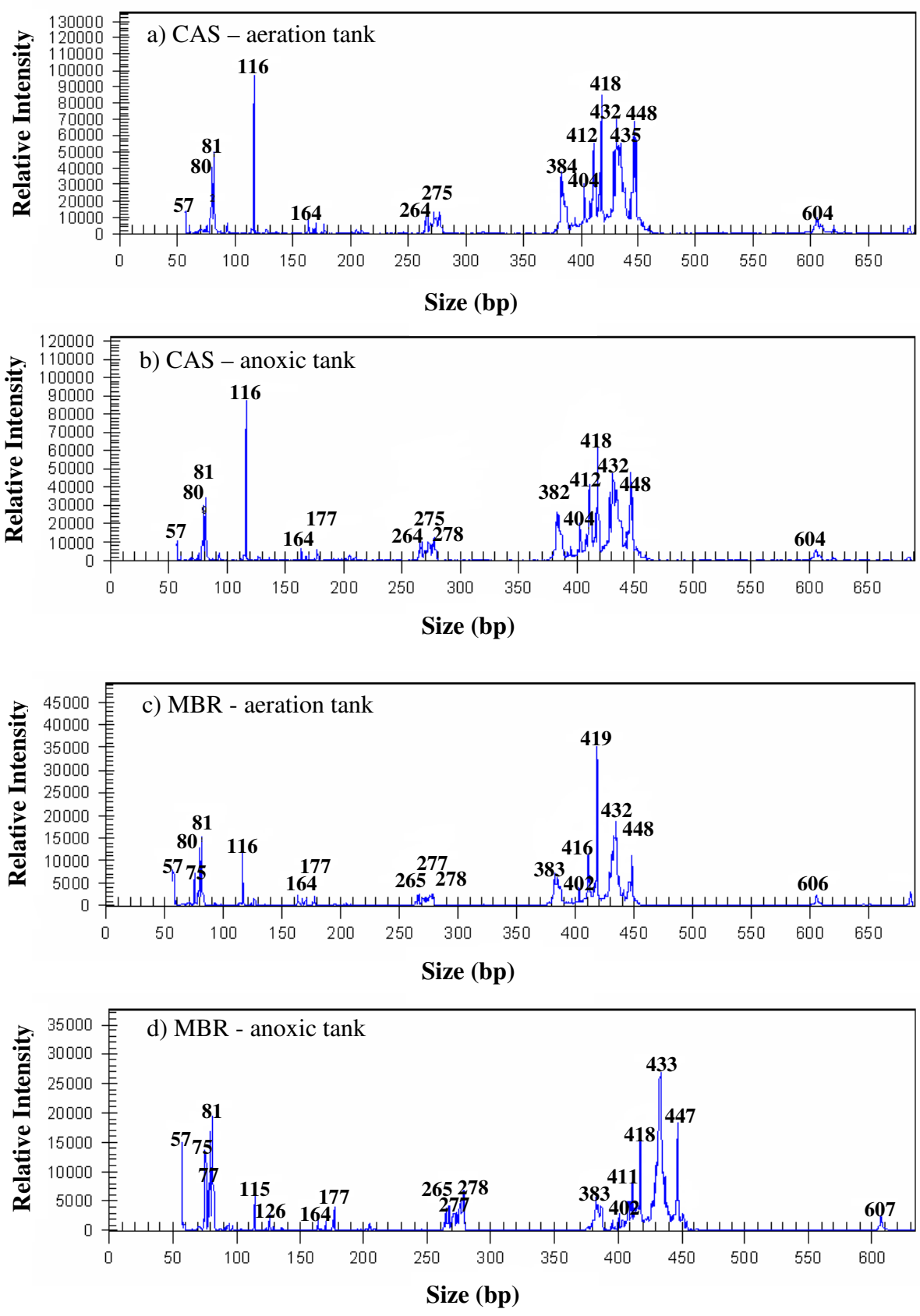


Figure 4.39 T-RFLP fingerprint profiles of aeration and anoxic tanks of CAS and MBR at 8-h HRT using bacteria-specific primers and RspI enzyme (50% sewage addition).

In the last operating condition of the study, 50% sewage was added to the post-treatment systems. Species at 288 bp peak that was present in all the rest of the operating conditions was still domineering in both CAS and MBR. However, the intensity of the species at 286 bp had increased in the last operating condition.

As compared to the previous operating condition, bacteria species found in CAS had reduced as shown in Figures 4.39a and 4.39b. However, Figures 4.39c and 4.39d show a different trend, where bacteria species in MBR increased.

From the T-RFLP study, the microbial communities in the CAS and MBR were found to vary in different operating conditions. Different domineering species would prevail under certain operating conditions, but it was noted that many similar species were found in the systems throughout the study.

CHAPTER FIVE CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The study for the post-treatment of anaerobic effluents was carried out in 2 phases with different focuses. Experimental analyses were done to compare the performance between the post-treatment of effluent from UASB, AF and anSBR by CASs and MBRs under different operating conditions. Conclusions were drawn based on the experimental results, with reference to the objectives highlighted in Chapter 1.4 of this study. The research findings are summarized as follows:

5.1.1 Phase 1 - Organic removal and nitrification performance

1. All CAS and MBR systems were able to produce effluents of consistently good quality (less than 50 mg/L in tCOD and less than 10 mg/L in tBOD) that were able to meet the discharge requirement to controlled watercourse (tCOD < 60mg/l and tBOD < 20mg/l) of Singapore (NEA, 2005). MBRs outperformed CAS for both COD and BOD removal as performance of MBRs are independent of the settleability of biomass.

2. MBR was able to achieve complete solid-liquid separation, therefore no SS was observed in the effluent of MBR. The CAS were able to achieve effluent SS

concentration of less than 30 mg/L to meet Singapore standard for discharge to controlled watercourse (NEA, 2005)

3. The ratios of COD/BOD were found to be above 3, suggesting that biodegradation could be slow. Despite this, both CASs and MBRs were still able to produce good quality effluents.

4. A near complete nitrification performance was observed most of the time for all CASs and MBRs operated at both 8- and 4- h HRTs.

5. Severe membrane fouling was not observed throughout the study at 8 h HRT. Noticeable membrane fouling was observed for MBR operated at HRT of 4 h treating effluent from the UASB. However, similar observation was not found for the other 2 MBRs treating effluents from AF and anSBR.

5.1.2 Phase 2 - Nitrogen removal performance

1. CASs could achieve more than 85% tCOD removal while more than 90% removal was achievable by MBRs for all the 3 different percentage of sewage additions.

2. The nitrogen removal efficiency had improved tremendously from 20% to approximately 70% with the introduction of anoxic tanks for post-treatment systems treating the UASB and AF effluents. However, only about 50% removal was achieved in post-treatment of the effluents from the anSBR.

3. TN removal efficiencies for post-treatments of the effluents of the UASB and AF were similar to the operating condition when 0% of raw sewage was added. However, TN removal efficiencies of the post-treatment of the anSBR effluent improved tremendously by 16 and 19% for the CAS and MBR, respectively.

4. More than 70% TN removal efficiencies were achieved in all the post-treatment systems after addition of 50% sewage. However, the nitrification performance deteriorated with traces of NH_4^+-N found in effluents of both the CASs and MBRs.

5. To optimise the post-treatment process for anaerobic effluent, 50% raw sewage addition was the most ideal as the removal performances for nitrogen and organics were relatively high. In addition, it reduced the capacity of the pre-treatment while maximised the use of aeration in the post-treatment.

5.2 Recommendations

1. T-RFLP was performed to investigate the dynamics of microbial community within the systems. However, the microorganisms could not be identified by using this analysis alone. Therefore, other molecular techniques should be used in conjunction with T-RFLP to identify the microorganisms that are responsible for the biological processes.

2. Nitrification performance was not satisfactory during the study of denitrification with 50% sewage addition. Detailed study should be performed to investigate the causes for the inhibition of nitrification.

3. Phosphorous removal should be investigated in future study.

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